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1. INTRODUCTION

These biosafety policies of the Kansas City University of Medicine and Biosciences (the “**University**”) regulate the use of recombinant DNA molecules and other biohazardous materials in both the research and teaching laboratories of the University. The aim of these policies is to ensure that such regulated materials are used safely and in compliance with the *National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* (See Appendix I of this Policy) and the NIH and Center for Disease Control’s (CDC), *Biosafety in Microbiological and Biomedical Laboratories, Edition 5 (Biosafety Manual)* (See Appendix J of this Policy). These policies are in place to protect students, faculty, staff, the public, and the environment from exposure, and resulting consequences, to recombinant DNA molecules and biohazardous material the University uses during research and teaching. The primary and overriding goal is safety, with the ancillary aim of doing so without unnecessarily hindering research and teaching activities.

1.1 Definitions

- A. **Biohazardous Materials:** Any microorganism, or infectious substance, or any naturally occurring, bioengineered, or synthesized component of any such microorganism or infectious substance, capable of causing: 1) death, disease, or other biological malfunction in a human, an animal, a plant, or another living organism; 2) deterioration of food, water, equipment, supplies, or material of any kind; or 3) harmful alteration of the environment. These include, but are not limited to: Certain bacteria, fungi, viruses, rickettsia, protozoa, parasites; recombinant products; toxins of biological origin; allergens; cultured human or animal cells and the potentially infectious agents these cells may contain; viroids and prions; other infectious agents as outlined in applicable laws, regulations and guidelines.

Examples include all materials containing recombinant DNA; transgenic animals or plants; human, animal or plant pathogens; biological toxins (such as tetanus toxin); human blood and certain human body fluids; select agents; high consequence livestock pathogens and toxins; and human or primate cell cultures.

- B. **Biosafety Manual:** NIH and Center for Disease Control’s (CDC), *Biosafety in Microbiological and Biomedical Laboratories, Edition 5*. (See Appendix J of this Policy).

- C. **Infectious Agent(s):** Human, animal, and plant pathogens (bacteria, parasites, fungi, viruses, prions).

D. NIH Guidelines: *National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules (2011)* (See Appendix I of this Policy).

E. Non-Biohazardous Materials: Biological materials not usually infectious. Examples are non-pathogenic microorganisms, subviral agents, viruses, bio material unlikely to contain infectious agents, exempt recombinant DNA molecules under the *NIH Guidelines*, environmental samples unlikely to have infectious agents, and biologically-derived non-toxic molecules.

F. Principal Investigator: Encompasses both lead investigator of research project and instructors/course supervisors in teaching labs.

G. Recombinant DNA: Molecules constructed outside of living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or molecules that result from their replication (*NIH Guidelines*, 2002). Recombinant DNA molecules are considered biohazardous unless exempt from the *NIH Guidelines*. Examples are recombinant DNA formed by transfer of drug resistant trait to microorganisms that do not acquire the trait naturally; designed for use in human gene transfer; contains genes for the biosynthesis of toxic molecules lethal for vertebrates at a median lethal dose (LD50) of less than 100 ng/kg body weight; is designed for generating transgenic plants or animals; or contains infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of helper virus.

H. **Note: The terms "research" and/or "research activities" as used in this policy also encompass teaching and/or teaching activities at the University. Likewise, the terms "principal investigator(s)" and/or (PI(s)) encompasses, when applicable, instructor(s) for a teaching laboratory or teaching activity involving research.

1.2 Abbreviations

- A. **APHIS:** Animal and Plant Health Inspection Service
- B. **BSC:** Biological Safety Cabinet
- C. **BSL:** Biosafety Level
- D. **BSO:** Biological Safety/Biosafety Officer
- E. **CDC:** Centers for Disease Control and Prevention
- F. **DOT:** Department of Transportation
- G. **HHS:** United States Department of Health and Human Services
- H. **IBC:** Biological Safety Committee
- I. **IO:** Institutional Official (the VPR)
- J. **NIH:** National Institutes of Health
- K. **OBA:** Office of Biotechnology Activities
- L. **ORSP:** Office of Research and Sponsored Programs (formerly

- Department/Division of Research)
- M. **PI:** Principal Investigator
 - N. **rDNA:** Recombinant DNA
 - O. **USDA:** United States Department of Agriculture
 - P. **VPR:** Vice President for Research (also the IO)

1.3 Materials and Activities Covered

All work with recombinant DNA, whether classified as exempt or non-exempt according to the *NIH Guidelines* and work with any biohazardous materials (See Section 1.1(A) of this Policy). Any research involving recombinant DNA molecules (See Section 1.1(G) of this Policy) must be registered with the University's IBC, regardless of whether such research is exempt under NIH Guidelines (See Section 13 of this Policy for IBC Procedures). **BSL-4 research is not currently allowed at the University.**

1.4 Roles and Responsibilities

Effective implementation of the University Biosafety Program requires the effective cooperation of several individuals and groups within the campus community. Individuals falling under these policies may fall into more than one category.

1.4.1 Principal Investigators

The PI is vested with the primary responsibility for ensuring safe performance of research activities within a research or teaching lab on the University's campus. For teaching laboratories that are associated with courses, the designated instructor/lab supervisor shall have the same responsibilities as the PI.

The PI's **minimal** responsibilities are the following:

- A. Reading and being knowledgeable of this policy and the procedures and guidelines contained within.
- B. Being familiar with principles and procedures appropriate for the safe use of the specific recombinant DNA molecules and biohazardous materials used in his or her research and following/applying those principles and procedures.
- C. Conducting a risk assessment and determining the appropriate biosafety level(s) for the materials to be used in his or her lab.
- D. Using risk assessment to determine lab procedures, containment equipment, personal protective equipment, and facilities that are

suitable for the biosafety level(s).

- E. Making sure lab facilities are maintained, equipment is in safe working condition, and biohazard warning signs are posted where appropriate, including lab entrances, and freezers, refrigerators, and any other containers holding recombinant DNA molecules or other biohazardous materials. Ensuring that animal rooms involving recombinant DNA-modified organisms used with animals have postings on the associated risks. For details on signage requirements, see Exposure Control Plan Section 6 (See Appendix C of this Policy), Hazard Communication Plan, and NIH Guidelines Appendices P-II-B-1-f, P-II-C-1-f, P-II-D-1-f, P-II-D-1-f, Q-11-B-1-C, Q-II-C-1-C, and Q-II-D-1-C.
- F. Determining the minimum Personal Protective Equipment for lab staff and ensuring personnel are trained on the proper use of PPE and are in fact properly using PPE. Supervising the safety performance of the lab staff, which includes monitoring Personal Protective Equipment compliance. See NIH Guidelines IV-B-7-e-(1) and NIH Guidelines Appendix G.
- G. Consulting the *NIH Guidelines* that apply to the type of research to be conducted and indicating such on the protocol application for IBC review.
- H. Prior to beginning recombinant DNA or Biosafety Level 1-3 research, submitting research protocols for review by, and registration with, the Institutional Biosafety Committee (See Section 13 of this Policy for IBC procedures), unless the research activities are authorized by the *NIH Guidelines* to the IBC simultaneously with beginning research (See Section III-E of the NIH Guidelines. PIs are also responsible for submitting to the IBC subsequent changes to such protocols.
- I. Submitting protocol renewal and amendment forms (See Appendix G) as well as incident reports to the IBC as required (See Sections 13.6(c)(4) and 13.11(a)-(c) for more on incident reports).
- J. Ensuring all lab personnel are appropriately trained for working with biohazardous materials and/or recombinant DNA, use of safety equipment, and the *NIH Guidelines*. This includes ensuring personnel have undergone the University's required training program as outlined in Appendix A of this Policy, as well as any additional lab-specific training needed to be in compliance with the *NIH Guidelines*. PIs are responsible for keeping records of training that individual personnel have undergone and submitting such to

the IBC and ORSP. For laboratory specific training, there is no specific form for documenting lab-specific training, but PIs must ensure that in whatever format is used for documentation includes information about (1) who was trained, (2) where they were trained, (3) the date of training, (4) the content/subject/tasks of the training, (5) a signature by the PI who gave the training, (6) the signature of the individual who underwent the training, and (7) the role the trained individual will serve in the research project.

- K. Prior to initiating research, making available to lab staff, (1) the protocols that describe the potential biohazards and the precautions to be taken, (2) instructing and training lab staff in (a) the practices and techniques required to ensure safety and (b) the procedures for dealing with accidents, and (3) informing lab staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations or serum collection).
- L. Supervising the safety performance of the lab staff to ensure that the required safety practices and techniques are employed.
- M. Correct work errors and conditions that may result in the release of recombinant DNA materials.
- N. Investigate and report any significant problems pertaining to the operation and implementation of containment practices and procedures in writing to the Biological Safety Officer (where applicable), Greenhouse/Animal Facility Director (where applicable), Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities (if applicable) (reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax)).
- O. Correct work errors and conditions that may result in the release of recombinant DNA materials.
- P. Ensure the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., purity and genotypic and phenotypic characteristics).
- Q. Complying with reporting requirements for gene transfer experiments conducted in compliance with the *NIH Guidelines* (See Appendix M-I-C, *Reporting Requirements*).
- R. Reporting newly identified select agents immediately to the IBC

and/or Biosafety Officer.

- S. Timely requesting collection of biohazardous waste.
- T. Making available reference information on biological hazards and that research personnel knows how to use such references.
- U. Knowing and implementing when required the proper procedures to use in the event of an emergency or spill/release.
- V. Complying with shipping requirements for rDNA and biohazardous materials.
- W. Reporting all significant violations, spills, releases, injuries or illnesses use of materials during research to the IBC and/or Biosafety Officer.
- X. Reporting all exposures to biological agents or recombinant DNA, theft or loss of biohazardous material, and incidents that warranted emergency response to the IBC.
- Y. Cooperating with Annual Biosafety Inspections of the lab, including those by the Biosafety Officer.

1.4.2 Research Personnel

Research personnel are responsible for:

- A. Completing all University requirements for approval to work in the lab and making sure all work performed in the lab complies with University policy, NIH, CDC, OSHA and any other guidelines the research activities fall under.
- B. Learning and following the standard operating procedures for the lab. Familiarizing self with potential hazards, infectious agents being used, and emergency procedures.
- C. Help keep the lab facility in good, operable condition.
- D. Report to the PI all medical restrictions, reportable illnesses, and any potential exposure. Report all conditions that are irregular.
- E. Follow the University training program as outlined in Appendix A of this Policy and as well as training from PI to ensure proficiency in appropriate microbiological practices.

- F. Completing medical surveillance requirements, if applicable.
- G. Following instructions given by PI regarding keeping the lab maintained and operating functionally.

1.4.3 Deans, Directors, and Department Heads

The Dean, Director or Department Head are responsible for:

- A. Ensuring all persons under his or her jurisdiction have access to the University's biosafety manual.
- B. Ensuring PI has taken required training for the research activity to be performed.
- C. Ensuring availability of facilities are appropriate for containing biohazardous materials and sufficient to enable the PI to comply with University policies.
- D. Ensuring the IBC reviews research activity involving biohazardous materials.
- E. Ensuring accidents/incidents are reported to Biosafety Officer.
- F. Ensuring all personnel who are eligible are assigned to occupational health program that is appropriate.
- G. Assisting in eliminating known unsafe practices.

1.4.4 Institutional Biosafety Committee

The IBC is a committee appointed by the Chief Executive Office in coordination with the VPR. See Section 13 of this Policy for procedural information regarding the IBC. The IBC's responsibilities include, but are not limited to:

- A. Review research protocols involving recombinant DNA and materials that are biosafety level 2 (infectious agents, biological toxins, and other biohazardous materials) and higher (collectively referred to as "covered research activities) to ensure they comply with the *NIH Guidelines* and *The Select Agent Rule*, reviewing protocols for biosafety concerns, the *CDC Manual* and University policy.
- B. Overseeing implementation of University biosafety procedures.
- C. Independently assessing containment levels required by the *NIH Guidelines* and/or *CDC Manual* for proposed research.

- D. Assessing the facilities, procedures, practices, and training and expertise of personnel involved in covered research activities for compliance with federal and state guidelines and law.
- E. Ensuring that all aspects of Appendix M of the *NIH Guidelines* have been appropriately addressed by the Principal Investigator.
- F. Ensuring no research participant is enrolled (defined in Section I-E-7 of the *NIH Guidelines* in a human gene transfer experiment until the RAC review process has been completed (See Appendix M-I-B of the *NIH Guidelines* for RAC review requirements), IBC approval from the clinical site has been obtained, Institutional Review Board approval has been obtained, and all applicable regulatory authorizations have been obtained.
- G. For human gene transfer protocols selected for public RAC review and discussion, considering the issues raised and recommendations made as a result of this review and considering the PI's response to the RAC recommendations.
- H. Ensuring final IBC approval is not granted until after the RAC review process has been completed.
- I. Ensuring compliance with all surveillance, data reporting, and adverse event reporting requirements set forth in the *NIH Guidelines* and this University policy.
- J. The University's Chief Executive Officer has authorized the IBC to disapprove of or suspend research in noncompliance with policies and procedures described in the *Biosafety Manual*, *NIH Guidelines* and/or University policy, and the IBC has the responsibility to do so. Likewise, if a lab inspection results in a determination that lab procedures are not being followed, research may be halted until actions are taken to correct deficiencies.
- I. Notifying the PI of the results of the IBCs review and approval or disapproval. In the case of disapproval, notifying the PI of the reason for the disapproval and giving the PI an opportunity to respond.
- J. Lowering containment levels for certain experiments as specified in Section III-D-2-a of the *NIH Guidelines*
- K. Setting containment levels as specified in Sections III-D-4-b and III-D-5 of the *NIH Guidelines* when applicable.
- L. Periodically reviewing covered research conducted at or sponsored by the University to ensure compliance with the *NIH Guidelines*.

- M. Adopting emergency plans covering accidental spills (See Appendix B of this Policy, NIH Lab Safety Monograph for reference) and personnel contamination resulting from recombinant DNA research.
- N. Reporting significant problems or violations of the *NIH Guidelines* and any significant research-related accidents or illnesses to the Institutional Official, who shall file a report to NIH and the OBA within 30 days. Unless the IBC determines a report has already been filed to the NIH and the OBA by the PI.
- O. For recombinant DNA work not explicitly covered by the *NIH Guidelines*, not approving initiation of such experiments until NIH establishes the containment requirement.
- P. Ensuring compliance with the University's OSHA Exposure Control Plan (Appendix C of this Policy), when applicable.
- Q. The IBC is responsible for reporting incidents to the Institutional Official.

*Refer to Section 13 of this policy for IBC procedure.

1.4.5 Biological Safety Officer

- A. The Biological Safety Officer is a faculty member knowledgeable of biosafety issues who is appointed by the Chief Executive Officer. The Biological Safety Officer shall always be a member of the IBC. The University shall have a Biological Safety Officer with the following responsibilities include but are not limited to:
 - 1. Periodically inspecting lab facilities to ensure that laboratory standards are rigorously followed.
 - 2. Investigating every accident with possibility of escape and/or exposure to materials that are potentially infectious or toxic.
 - 3. Reporting to the IBC and the University any significant problems, violations of the *NIH Guidelines*, and any significant research-related accidents or illnesses of which the Biological Safety Officer becomes aware unless the Biological Safety Officer determines that a report has already been filed by the Principal Investigator.
 - 4. Developing emergency plans for handling accidental spills and personnel contamination and investigating laboratory accidents involving recombinant DNA research.

5. Ensuring that following of decontamination procedures after spills and/or breakage involving biohazardous materials and keeping record of such accidents and incidents, ensuring that materials and equipment are being appropriately decontaminated and monitoring infectious waste disposal after treatment for safety.
6. Providing advice on laboratory security.
7. Providing advice on laboratory security.
8. Providing technical advice to Principal Investigators and the IBC on research safety procedures.

Note: See the *Laboratory Safety Monograph* (Appendix B of this Policy) for additional information on the duties of the Biological Safety Officer.

9. Complying with all IBC procedures, Section 13 of this Policy (including 13.10(A)).
10. Serving as a member on the University's IBC.

1.4.6 Office of Research and Sponsored Programs

The ORSP has the responsibility for all official communication between the University and federal and state agencies such as NIH, NSF, the USDA, HHS, etc. The ORSP maintains the IBC website, oversees and administers training modules and examinations required of PIs and other research personnel. The ORSP is also responsible for maintaining documentation of research personnel training.

1.4.7 Vice President for Research

The responsibility for assuring comprehensive programs on campus for safe handling of infectious agents, recombinant DNA molecules, and all other biohazardous materials at the University rests with the Executive VPR. The VPR is responsible for administration of the University's Biosafety policies. This includes appointing members to the IBC in coordination with the Chief Executive Officer, appointing the Biological Safety Officer, and approving all IBC-recommended policies and procedures. As IO, the VPR is responsible for all IO duties, including providing final incident reports to the NIH OBA.

2. Biological Risk Assessment

Before submitting a protocol for review by the IBC, all PIs are required to conduct a risk assessment of the proposed research and materials to be used (see Pg. 41 of the *Biosafety Manual*). The CDC and NIH require such risk assessments to protect the health of lab personnel and the public. The PI's goal during risk assessment is to identify potential hazards and risks involved with the planned research or teaching activities, then to develop and implement standard operating procedures and practices to minimize those risks to the lowest possible level. The PI must conduct a risk assessment of each of his or her research and/or teaching labs. After the risk assessment, the PI must assign the appropriate Biosafety Level (BSL) to the agents that will be used. Additionally, through risk assessment the PI must assess several factors that contribute to potential risk, including, but not limited to, the hazardous characteristics of the lab procedures to be used; the training, experience and habits of lab personnel; and the adequacy of the lab facility and equipment. The following policy outlines these elements. If the *Biosafety Manual*, *NIH Guidelines* and any other mentioned reference materials do not account for the type of agents or nature of the lab procedures that are contemplated for the research, the PI is responsible for using the best information available. In all risk assessments, the PI should consult available scientific references along with, and not in place of, his or her professional judgment.

Note: Biological Safety Level 4 and Animal Biological Safety Level 4 Research is Currently Prohibited at the University.

Note: For any activity involving a select agent or toxin, in addition to risk assessment, developing lab safety procedures, and getting approval from the IBC, all federally mandated applications to applicable federal agencies must be approved before beginning the activity. These select biological agents and toxins are listed in 7 CFR 331, 9 CFR 121, 42 CFR 73, and on the HHS and USDA select agents and toxins list online. Select Agents are also discussed in Section Six and listed in Appendix F of the *Biosafety Manual*. Also see the U.S. Government's Select Agent list for reference. A list of Select Agents can be found in Appendix F of this Policy.

2.1 Risk Assessment Procedure

The risk assessment process consists of the following five steps. Principle investigators should refer to Section 2 of the *Biosafety Manual* for detailed guidance on conducting these steps.

- A. Identify agent hazards and perform an initial assessment of risk.
- B. Identify Laboratory Procedure Hazards

- C. Determine Appropriate Biosafety Level and Select Additional Precautions as indicated by the Risk Assessment.
- D. Evaluate the Proficiencies of Staff Regarding Safe Practices and Integrity of Safety Equipment.
- E. Review the Risk Assessment with IBC and a biological safety expert and/or consultant when determined necessary by the PI, IBC, or University.

2.2 Risk Assessment Guidance

A. Guidance For the Procedural Steps Outlined Above in Section 2.1 of this Policy.

1. Identify agent hazards and perform initial Assessment of risk

For this initial phase of the risk assessment, PIs should begin by consulting the *Biosafety Manual*, Section 2 (beginning pg. 9), including Table 1 (pg. 10). The process of the risk assessment for hazardous characteristics of an agent is explained in pages 9-13 and 16. PIs should also consult Section 4 (pg. 30), **unless lab work is to be conducted with animals**, in which case consult Section 5 (page 60) and Table 3 (page 103) instead; Table 2, *Summary of Recommended Biological Safety Levels for Infectious Agents* (pg. 59); and the *Agent Summary Statements*, Section 8, Page 123. Additionally, for research involving recombinant DNA, see Section 2.3 of this policy. For research involving HIV, Hep B or other bloodborne pathogens, additionally see Section 2.4 of this policy. Page 13 of the *Biosafety Manual* offers additional detail for risk assessment for cell cultures and furthermore, Appendix H provides guidance for work with human, non-human primate, and other mammalian cells and tissues.

2. Identify Lab Procedure Hazards

Pages 14 and 17 of the *Biosafety Manual* explain the process of identifying laboratory procedure hazards.

3. Determine Appropriate Biosafety Level and Select Additional Precautions as indicated in Risk Assessment

Page 15 and 18 of the *Biosafety Manual* explain the process of determining the appropriate biosafety level and additional precautions.

4. Evaluate Proficiencies of Staff Regarding Safe Practices and Integrity of Safety Equipment.

Page 18 of the *Biosafety Manual* provides an overview of evaluating staff and safety equipment.

5. Review the Risk Assessment with a Biosafety Professional, Subject Matter Expert, and the IBC.

Page 19 of the *Biosafety Manual* provides more information about reviewing the risk assessment with other parties.

2.3 Additional Risk Assessment Guidance for Research Involving Recombinant DNA (for Both Animal and Non-Animal Lab Work)

In addition to the resources contained in Section 2.2 of this policy, when beginning a risk assessment for recombinant research, Principal Investigators should also consult the *NIH Guidelines*, Section II-A, *Risk Assessment*, and *NIH Guidelines*, Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*.

After this initial assessment of agents, to determine and document proper containment measures, the PI shall consult the following guidance in the *NIH Guidelines*: Section II-B, *Containment*; Section V-B, *Footnotes and References of Sections I-IV*), Appendix G, *Physical Containment*, Appendix I, *Biological Containment*.

For research involving plants, PIs shall additionally reference Appendix P of the *NIH Guidelines*. For research involving animals, PIs shall additionally reference Appendix Q of the *NIH Guidelines*.

2.4 Additional Risk Assessment Guidance for Research Involving Bloodborne Pathogens

For research or teaching activities involving bloodborne pathogens, PIs shall additionally consult the University's OSHA Exposure Control Plan when assessing risk and developing lab safety procedures.

3. Development of Lab-Specific Safety/Standard Operating Procedures

After completing an appropriate risk assessment as described in Section 2 of this policy and Section 2 of the *Biosafety Manual*, investigators must develop lab safety/standard operating procedures that minimize or eliminate the risks determined in the risk assessment. For most BSL-1 labs, standard operating procedures need only include the standard microbiological practices contained on page 30, of the *Biosafety Manual*, or for work with animals, page 61 of the *Biosafety Manual*, unless the PI, after conducting a risk assessment, determines

additional safety measures should be integrated in the lab safety procedures. The standard microbiological practices, or when applicable, animal microbiological practices, apply to all research, but additional practices are recommended for each ascending biological or animal biological safety level.

A. Guidance for Developing Lab Safety/Standard Operating Procedures as Required in Section 3 of this Policy Above.

In addition to consulting his or her own risk assessment of the contemplated research, PIs should consult the *Biosafety Manual* for recommended microbiological practices, safety equipment (both primary barriers and personal protective equipment), and laboratory facilities (secondary barriers) to integrate in their lab's standard operating procedures. The *Biosafety Manual* is not meant to be an exhaustive source for developing lab procedures. PIs should consult the best information available for their research as well as their professional judgment. **For recombinant research, PIs must consult Appendix G of the NIH Guidelines for developing procedures**, when applicable based on the type of recombinant research. When developing lab procedures for an animal lab, PIs must consult both the *Biological Safety Manual* and the *PHS Guide for the Care and Use of Laboratory Animals, 8th Edition*. Be sure to include any other necessary content such as decontamination, Exposure Control Plan, Occupational health, chemical hygiene procedures, biological spill response plan, etc.

The *Biosafety Manual* offers the following sections for guidance:

1. Procedures for Research Not Involving Animals

For microbiological research not involving animals, PIs should consult pages 30-33 of the *Biosafety Manual*, for BSL-1 research, pages 33-38 for BSL-2 research, pages 38-42 for BSL-3 research. BSL-4 research is not currently allowed at the University.

2. Procedures for Research Involving Animals

For microbiological research involving animals, PIs should consult pages 61-67 of the *Biosafety Manual* for Animal BSL-1 (ABSL-1), pages 67-73 for ABSL-2, and pages 73-82 for ABSL-3. ABSL 4 is not currently allowed at the University. Additionally, PIs must develop lab procedures that comply with the PHS Guide for the Care and Use of Laboratory Animals, 8th Edition.

3. Biological Safety Cabinets

When applicable, to assist in selecting proper biological safety cabinets and developing proper procedures, PIs should consult Appendix M of the *Biosafety Manual* beginning on page 290 and Table 1, *Selection of BSC through Risk Assessment*, page 311.

4. Safety Equipment: Proper Use, Maintenance

4.1 PIs are responsible for being familiar with, and instructing all lab personnel on the proper use of all laboratory equipment including Incubators, centrifuges, autoclaves, emergency, biological safety cabinets, and all other equipment, including what to do in the event of a spill involving the equipment.

4.2 Biological Safety Cabinets (BSC)

A. Overview

Biological Safety Cabinets are a form of primary containment aimed at protecting personnel, the environment, and the product, depending on the type of BSC. BSCs are classified into three types, Class I-III. Class III BSCs are further sub-classified as Type A or B. A BSC contains a High Efficiency Particulate Air Filter (HEPA) to remove particles from the air stream. A HEPA filter does not remove vapors or gases.

National Sanitation Foundation Standard # 49 – 2007 governs all models of Class II cabinets (A1, A2, B1, B2). On-site field testing as per NSF/ANSI Standard 49-2007 Annex F plus Addendum #1 must be performed by qualified personnel. The Standard provides information to assist with the frequency of, qualifications for and procedures for testing. Each of the three classes of BSCs require **annual testing and recertification**.

B. Selection, Installation, and Use of Biosafety Cabinets (BSC)

PIs are responsible for being familiar with and practicing proper selection, installation, and use of BSCs. Appendix A, beginning on page 290 of the *Biosafety Manual*, provides guidance of which PIs must be knowledgeable. A description of the different classes of BSCs begins on page 292. Issues relating to lab hazards and risk assessment are addressed on page 298. Guidance for working with chemicals, as well as possible radiological hazards, within BSCs begins on page 298. Proper work practices and procedures begin on page 300. Possible hazards are addressed on page 302. BSC decontamination information is summarized on page 304. PIs, BSOs, and the University are responsible for working together to ensure the facility and engineering requirements beginning on page 305 are properly met and maintained. The University is responsible for obtaining annual certification from the appropriate manufacturers and/or certifying bodies. Table 1, *Selection of BSC through Risk Assessment*, page 311 of the

Biosafety Manual is a helpful reference during the risk assessment phase described in Section 2 of this Policy.

5. Spills

5.1 Reporting

5.1.1 Time Frames for Reporting

Any significant problems with or violations of the *NIH Guidelines* for work with recombinant DNA molecules or any significant research-related accident and illness must be reported to the appropriate institutional official and the NIH/OBA within 30 days (*NIH Guidelines*, Sec. IV, B(2)(b)(7)). Spills and accidents that result in an exposures or loss of containment involving materials in BSL-2 require immediate reporting to the DOR and Biosafety Officer. Spills, overt or potential exposures and loss of containment involving materials in BSL-3 also require immediate reporting to the DOR and Biosafety Officer. Any spills, accidents, or breach of containment involving recombinant DNA materials must be reported immediately to the DOR and Biosafety Officer. The PI is responsible for reporting the above incidents to the DOR and Biosafety Officer. The DOR will help in determining if an event requires reporting to the NIH/OBA. The designated official for the University will report any incidents to NIH OBA. The IBC will direct a follow up investigation if warranted.

5.2 Biological spill kit

A biological spill kit must be located in every lab handling biological agents. The spill kit should easily accessible in a central location. Spill kits must be checked monthly to ensure contents are complete. A sample list of items to include in a spill kit includes the following. Note this list is not exhaustive for all laboratories, procedures and organisms, and must be modified when the PI deems necessary to enhance protections.

- Plastic container with closable lid to contain the contents of the spill kit
- Absorbent towels
- Forceps to pick up sharps or glass
- Biohazard bags
- Household bleach or appropriate disinfectant for the organism being manipulated
- Container to mix dilute disinfectant solution, one gallon jug
- Appropriate PPE (gloves, goggles, tyvek jump suit, booties, specific PPE to protocols)
- Comet®, Ajax® or the like. Can be used to contain small spills. The chlorine in these products can be corrosive.
- Plastic scoop, plastic scraper and or autoclavable/disposable broom and dust pan

5.3 Biological Spill Response Plan

It is the responsibility of the PI to develop a biological spill plan for each of his or her labs. The plan must be appropriately customized for the agents and research in the lab for which it is developed. The spill response plan should be available to both personnel working in the laboratory and visitors. Also, the spill response plan must be posted visibly in the lab. PI's risk assessment(s) should be included in the plan. The following elements should be included in a biological spill response plan:

- Principal investigator's/lab director's name and current contact information. Lab staff and current contact information.
- Office of Research and Sponsored Programs contact information (Asst. Dir. of Research Compliance, 816-654-7605, irb@kcumb.edu. contact information)
- Microbiological agents being manipulated in the lab. If necessary, can be exceptions due to select agent requirements (Consult CDC, USDA - 7 CFR 331, 9 CFR 121, 42 CFR 73 for guidance on select agents as well as, Section 2.2(A)(6) of this policy).
- Location/inventory of spill kit(s).
- Location of closest spill kit outside the lab.
- Decontamination plans.
- Data sheets for the microbiological agents and procedures to follow if potential for exposure exists.

5.4 Elements of a Biological Spill Risk Assessment

5.4.1 When developing a biological spill plan for a lab, the PI must first conduct a risk assessment of the factors that will affect the outcome of a spill. After identifying the risks, the investigator must evaluate whether equipment, procedures, the agent, etc. be changed in a way to reduce risk without eliminating the ability to carry out the research. If so, the PI must implement those measures. Identified risks must be communicated to all lab personnel. Consider the following factors when conducting a biological spill risk assessment:

1. The microbiological organism.
2. The risk group classification of the organism. See Section 2.2(A)(1) of this policy and Section 2 of the *Biosafety Manual*.
3. Route of infection? (mucous membrane exposure, aerosol, ingestion, etc.).
4. The volume manipulated.

5. Determine appropriate disinfectant for the agent, contact time for the disinfectant, and equipment considerations with the disinfectant (e.g., if bleach, be aware that can corrode equipment and requires wiping down with water after use).
6. Whether the spill would be contained or not (would it occur in a BSC, open lab, or open common spaces?)
7. Would there be potential for environmental contamination.
8. Are any glass or sharp objects used in the research activity?
9. What procedures will be used in the lab? Could they be modified in a way to reduce risk of spills/risks identified with spills?
10. Where would the work be performed?
11. Would biological material be transported outside the lab?

6. Exposure to Infectious Agent or Material/Emergency Procedures:

Before beginning research, the PI must integrate emergency procedures into the laboratory safety procedures. For bloodborne pathogens, the University's Exposure Control Plan shall be followed (Appendix C of this Policy). For exposure involving other infectious agents/materials, the PI must delineate procedures in cases of exposures involving intact skin; broken, cut, damaged skin, puncture wounds, eye exposure, and ingestion/inhalation. The lab procedures shall indicate an emergency contact number. Lab procedures must also instruct exposed personnel, when appropriate, to call 911 or seek medical advice from the Employee Health Clinic on campus. PIs wishing to have assistance in developing exposure/emergency procedures should contact the Assist. Dir. of Research Compliance at 816-654-7605 or irb@kcumb.edu.

7. Medical Surveillance (Available Vaccines/Treatments For Lab's Infectious Agent, Procedures To Follow, Symptoms To Monitor).

When applicable, PIs should consult the University's Occupational Health Program when developing their protocol and integrate the OCP into their lab procedures when appropriate. The IBC and IACUC will withhold approval when the appropriate lab personnel are not enrolled in the University's Occupational Health Program.

8. Decontamination and Disinfection

A. General

PIs are responsible for integrating effective decontamination and disinfection procedures into their standard operating procedures, as well as ensuring such procedures are taught to lab personnel and implemented in practice. Physical and chemical means of decontamination fall into five main categories: heat, liquid, chemical vapors, gases, and radiation. To select the proper method and tools, it is important to consider, for example, the following aspects:

1. Type of biohazardous agents, concentration and potential for exposure.
2. Physical and chemical hazards to products, materials, environment and personnel.

The *Biosafety Manual* offers guidance on decontamination and disinfection, and PIs are responsible for being in compliance with the *Biosafety Manual*. Guidance can be found in Appendix B, *Decontamination and Disinfection*, page 326 of the *Biosafety Manual*. PIs should be familiar with Table 1: *Descending Order of Resistance to Germicidal Chemicals*, page 330 of the *Biosafety Manual* and Table 2, *Activity Levels of Liquid Germicides*, page 333. Decontamination of large spaces can be found on page 331 of the *Biosafety Manual* and decontamination of surfaces on page 332. Page 334 of the *Biosafety Manual* covers special infectious agent issues.

B. Recombinant Materials

PIs working with recombinant materials shall additionally refer to any decontamination and disinfection guidance offered in the *NIH Guidelines*. The *NIH Guidelines* require that all recombinant DNA materials be appropriately decontaminated before disposal. Any and all recombinant DNA materials, including transgenic animal carcasses, must be decontaminated by autoclave treatment, chemical treatment, incineration or by any other acceptable means specific to those materials. The IBC must be notified in the event that the decontamination of rDNA materials cannot be met. See Appendix G-II-B-2-I and G-II-C-2-n of the *NIH Guidelines*.

C. Assistance

PIs wishing to have assistance with developing decontamination and disinfection procedures should contact the Assist. Dir. of Research Compliance at 816-654-7605 or irb@kcumb.edu.

9. Infectious Waste Disposal and Disinfection (Credit to University of Missouri-Kansas City 2009 Policy)

9.1 Authority

Infectious waste disposal is governed by the state of Missouri. Missouri has issued regulations governing infectious waste disposal, and those standards apply to the University, which resides in Missouri. For the purposes of this policy, biohazardous waste and infectious waste are considered the same. The standards for infectious waste disposal are in the Missouri Code of State Regulations, Title 10 – Department of Natural Resources, Division 89 – Solid Waste Management.

9.2 Responsibility:

Any person who generates infectious waste as defined in the Missouri Code of State Regulations is responsible for the safe disposal of that waste (see Section 9.3 of this policy for infectious waste definition). Infectious waste at the University is also considered biohazardous. Waste generated in any lab at the University that could be reasonably included in the below definitions (Section 9.3) of infectious waste must be considered waste that infectious/biohazardous waste.

9.3 Definition of Infectious Waste

In Missouri, a “small quantity generator” of infectious waste produces 100 kg or less per month (10 CSR 80-7.010(1)(A)(2). Under this definition, the University is a small quantity generator. According to 10 CSR 80-7010(1)(A)(2), infectious waste for small generators is defined under 19 CSR 20-20.010(21). Infectious waste is defined as:

...waste capable of producing an infectious disease. For a waste to be infectious, it must contain pathogens with sufficient virulence and quantity so that exposure to the waste by a susceptible host could result in an infectious disease. Infectious waste generated by small quantity generators shall include the following categories:

(A) Sharps-all discarded sharps including hypodermic needles, syringes and scalpel blades. Broken glass or other sharp items that have come in contact with material defined as infectious are included;

(B) Cultures and stocks of infectious agents and associated biological-included in this category are all cultures and stocks of infectious

organisms as well as culture dishes and devices used to transfer, inoculate and mix cultures; and

(C) Other wastes-those wastes designated by the medical authority responsible (physician, podiatrist, dentist, veterinarian) for the care of the patient which may be capable of producing an infectious disease.

*The University is exempted from requiring an infectious waste processing permit under 10 CSR 80.7(C)(4).

10 Disposal of Infectious Waste Procedural Guidance

10.1 General

Infectious waste disposal at the University can be accomplished in two ways to meet the Missouri regulations. First, the waste may be inactivated and then disposed of in regular solid waste disposal. See Section 10.5 of this policy for information on inactivation. Secondly the waste may be disposed of by an outside company contracted to dispose of infectious waste. At the University the contracted infectious waste disposal company is **Bionomics, Inc.** The red biohazard tubs are stored in a designated area in most buildings on campus. The disposal of waste by **Bionomics, Inc.** requires that an account be put into place. The Director of Sponsored Programs and Research Compliance in the ORSP can assist in the establishment of an account. The cost associated with the account will be the responsibility of waste generator.

Inactivation of infectious/biohazardous waste at the University can be accomplished with a variety of methods. The accumulation, storage and transportation of any infectious waste must be in a secondary container. The container must be rigid and be puncture resistant. The use of a biohazard waste barrel, tub or other suitable container should be used while transporting waste for decontamination. The container should be labeled with the biohazard symbol. For solid and liquid waste the materials can be autoclaved. The length of the autoclave cycle is determined by the type of autoclave, the volume of materials, type of materials etc. The autoclave run must be recorded with the minimum of the following information; Type of materials autoclaved, weight or volume, autoclave cycle parameters, indicator results, name of autoclave operator, lab number, and phone number. This information should be retained by the individual or laboratory that generated the waste for documentation of inactivation of that waste. After sterilization the solid waste may be disposed of in the solid waste stream. The biohazard bags **MUST** be placed into a black bag or opaque bag or container prior to final disposal. Liquid waste may be disposed of into the sanitary sewer unless chemical or radiological hazards are present. Contact the Director of Sponsored

Programs and Research Compliance in the ORSP for guidance on final disposal if other hazards are present.

Sharps containers **MUST NOT** be disposed of in the solid waste stream. Sharps and sharps containers must be disposed of by the appropriate disposal company with whom the University has contracted. The sharps containers may be autoclaved prior to being deposited into the final disposal bins that are disposed of by **Bionomics, Inc.** as a best practice. The sharps containers must be securely sealed shut before disposal. Laboratory tape works well.

Many liquid infectious/biohazardous wastes may be inactivated by chemical decontamination/disinfection. The user of a liquid disinfectant must follow the directions of the disinfectant. The products information sheet will provide information on amount of disinfectant to use, contact time and final disposal will be outlined. The use of some disinfectants may produce a hazardous chemical waste that may need to be disposed of in the hazardous waste stream. Inactivated liquid waste can be then disposed of in the sanitary sewer system.

Any person or lab that generates infectious waste at the University has a responsibility to dispose of the waste in a way that is safe to anyone that will come in contact with the waste downstream. The waste must be disposed of in accordance with any and all state of Missouri requirements. Contact the Director of Research and Sponsored Programs in the ORSP with any questions concerning waste disposal.

10.2 Procedure

Any infectious waste generated must be disposed of in a manner outlined in the Missouri regulations. The generator of the waste is responsible for knowing and following the University's disposal of infectious waste policy. This section describes disposal of infectious waste as mandated by the Missouri Code of State Regulations, 10 CSR 80-7.010 (1).

- (A) All sharps shall be packaged in rigid, leak-resistant and puncture-resistant containers and sealed prior to disposal.

- (B) Infectious waste treated to render it innocuous may be disposed as a solid waste provided the treater certifies to the transporter, if other than the generator, and certifies to the sanitary landfill operator or processing facility operator that the waste has been rendered innocuous as required by Section 260.203, RSMo. (Note: Treated infectious waste is not required to be transported in accordance with the requirements of section (4) of this rule.)”

- (C) Certification of treated infectious waste, at a minimum, shall contain the following information: the name, mailing address, location (when different from the mailing address) and phone number of the office/facility treating the infectious waste; the printed name and the signature of the facility/office manager or person responsible for the treatment process; a brief description of the treated waste (sharps in metal containers, sharps in heavy gauge plastic containers, incinerator ash, laboratory wastes in autoclave bags); and a brief description of the method(s) of treatment (for example, steam sterilization, incineration, disinfection with bleach solution). In addition to these minimum requirements, the generator need only include a statement that the waste has been managed in accordance with the Missouri Solid Waste Management Law and rules and may legally be placed in a sanitary landfill. The certification shall be revised when changes in the operation of the office/facility result in a change to the information required by this paragraph. The University qualifies for an exemption from a processing facility permit under 10 CSR 80-7.010(c)(4).

11 Packaging of Infectious Waste: 10 CSR 80-7.010

- (A) Prior to transport, all infectious waste shall be placed in rigid or semi-rigid, leak-resistant containers clearly marked with the universal biohazard symbol prominently displayed and labeled Infectious Waste or Biohazard Waste and sealed. All containers shall be closed in such a manner as to completely contain all waste and the outside of the container shall be kept free of contamination. For the purpose of this rule, leak-resistant containers are defined as containers that are closable with a tight fitting lid and are leakproof on the bottom and sides. Containers meeting the requirements of 29 CFR 1910.1030 are acceptable.
- (B) Plastic bags. Plastic bags shall be tear resistant and leak resistant. Plastic bags shall not be used as primary containers for transportation of infectious waste. Infectious waste contained in plastic bags shall be placed within rigid or semi-rigid containers prior to transport.
- (C) Sharps Containers. Sharps shall be packaged in rigid, leak-resistant and puncture-resistant containers and sealed.
- (D) Glass Containers. Glass containers shall not be used as primary containers for transportation of infectious waste. Glass containers must be placed into a rigid or semi-rigid leak-resistant container and protected from breakage.
- (E) Reusable containers. Reusable containers shall be constructed of

either heavy wall plastic or noncorrosive metal. Each container shall be cleaned and sanitized before it is reused.

11.1 Tracking Documents, 10 CSR 80-7.010(3):

(A) Generators. The generator of infectious waste that is to be transported to a permitted infectious waste processing facility shall-

1. Prepare tracking documents which shall include, at a minimum, the following information:

a. The printed or typed name, mailing address, location (when different from the mailing address) and telephone number of the generator;

b. The printed or typed name and address of the designated facility which is permitted to process waste. The name and address of an alternate facility may also be designated to which the waste may be transported in the event an emergency prevents delivery of the waste to the primary designated facility;

c. The printed or typed name, address and Missouri Transporter identification number of the transporter's company, if other than the generator;

d. The quantity, in volume or weight, of waste to be transported;

e. A name and signature block for the transporter, if other than the generator; and

f. A name and signature block for the receiving facility;

2. Sign the tracking document by hand. The name of the generator signing the document shall also be printed or typed on the tracking document;

3. Obtain the handwritten signature of the transporter. If other than the generator, and date of acceptance on the tracking document. The name of the transporter signing the document shall also be printed or typed on the tracking document;

4. Retain one (1) copy of the tracking document with the signatures required in this subsection; and

4. Give the transporter the remaining copies of the tracking document.

11.2 Recordkeeping:

The appropriate University personnel shall ensure infectious waste disposal records are kept in accordance with 10 CSR 80-7.010(6) below:

All tracking documents, operating logs, quarterly fees reports, records, test results and process monitoring records shall be kept for a period of at least three (3) years. The period of record retention extends upon the written request of the [Missouri] department [of Natural Resources] or automatically during the course of any unresolved enforcement action regarding the regulated activity. These records shall be made available for inspection by the [Missouri] department [of Natural Resources] upon request.

12. Shipping Infectious Materials

12.1 Federal Regulation

All biohazardous materials that are regulated must be packaged and shipped in a manner compliant with current federal guidelines and codes. Appendix C, *Transportation of Infectious Substances*, page 336 of the *Biosafety Manual* offers guidance. Such shipping is governed by the United State Department of Transportation and international agencies and associations. In the United States, the United States Postal Service, the Department of Transportation, Department of Agriculture, Federal Aviation Administration, Centers for Disease Control and Prevention and various other agencies have some regulatory administration over the transport of infectious materials. Internationally the International Air Transport Association (IATA) and the International Civil Aviation Organization (ICAO) as well as the World Health Organization and the United Nations have input on the transportation of infectious substances and biological specimens. Because of the numerous and varied organizations involved a person who ships infectious materials should stay aware of the annual updates and changes made in the requirements to avoid shipping delays and fines.

The codified regulations in the United States falls under 49 CFR parts 171, 172, 173 and 175. 49 CFR is the codified federal regulation that regulated the transportation of any hazardous material in the United States. These regulations cover all forms of transportation. Fines can be leveled against an individual or organization that violates any part. Fines can range up to \$500,000 and imprisonment up to five years. Many federal agencies can level fines against a violator. Universities and their employees are not immune.

The shipper should consult with the relevant guidelines for updates and additions to the applicable regulation to ensure full compliance. International shipments entering and leaving the United States will require import export

documentation and further approvals. The approval process may be lengthy. Agents that have been included in the select agents list may require approval from CDC/APHIS and multiple other agencies **BEFORE** shipment. Plan ahead, the approval process can be lengthy.

12.2 Training requirements for Shipping

Anyone involved in shipping must be adequately trained. The training renewal frequency is every three years for the DOT and every two years for the IATA. For more information contact the Assist. Dir. of Research Compliance at 816-654-7605 or irb@kcumb.edu.

13. IBC Procedures

Generally, the IBC ensures research activities conducted at the University comply with the *NIH Guidelines* and The Select Agent Rule (list of Select Agents located in Appendix F of this Policy), reviewing protocols for biosafety concerns, and overseeing implementation of University biosafety procedures, updating the OSHA Occupational Exposure Plan, submitting reports as required to OLAW, documenting exposures. Detailed explanations of the IBC's responsibilities and conducting of meetings/reviews are outlined below.

13.1 Applicability

The IBC reviews all research protocols involving 1) recombinant DNA (Defined in Section 1.1(G) of this Policy) and 2) biological materials that are BSL-2 or higher (infectious agents, biological toxins, and other biohazardous materials) (Defined in Section 1.1(A) of this Policy), regardless of whether recombinant DNA is involved. The IBC also reviews 1) exposures to biological agents or recombinant DNA, 2) theft or loss of biohazardous material, and 3) all incidents that warranted emergency response and make a determination as to whether the protocol must be modified or the research halted. The IBC ensures research activities conducted at the University comply with the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (NIH Guidelines) and *The Select Agent Rule*, reviewing protocols for biosafety concerns, and overseeing implementation of University biosafety procedures.

Those materials falling within the scope of this policy must be registered with the IBC before the PI commences research. Funding source does not affect the applicability of this policy. Only University faculty members are allowed to submit protocols to the IBC.

13.2 Responsibilities

A. Principal Investigators

PIs wishing to conduct biohazardous research outlined in 1.1(A) of this policy must conduct a risk assessment as described in Section 2 of this policy and submit to the IBC an application (Appendix G of this Policy and available on IRBNet) to have any recombinant materials registered with the committee prior to beginning the research activity. PIs must also submit to the IBC renewal applications (Appendix G of this Policy and available on IRBNet) for ongoing research as described by this policy. PIs are responsible for submitting any changes in the protocol to the IBC as well as 1) exposures to biological agents or recombinant DNA, 2) theft or loss of biohazardous material, and 3) all incidents that warranted emergency response.

B. The IBC

The IBC is responsible for reviewing all research and teaching activities involving recombinant DNA conducted at or sponsored by the University for adherence with *NIH Guidelines*, regardless of funding source for the research. The committee is responsible for reviewing all research and teaching activities involving other biohazardous material conducted at or sponsored by the University for adherence to the *Biosafety Manual*, regardless of funding source for the research. The committee must approve those research and teaching activities involving recombinant DNA if they conform to the *NIH Guidelines* and other biohazardous materials if they conform to the *Biosafety Manual*. The committee must review research and teaching activities involving select agents and toxins conducted at or sponsored by the University, regardless of funding source, for adherence to the Select Agent Final Rule (a list of Select Agents is available in Appendix F of this Policy) and approving those activities if they comply. The committee must notify the PI in writing of the determinations made after review in regard to applications/protocols.

The IBC must also conduct risk assessments to determine appropriate levels of containment as defined in applicable regulations and assess lab facilities, procedures, practices, training, and expertise of involved personnel. The IBC is also charged with conducting periodic reviews to ensure compliance with federal, state and university laws, regulations, and guidelines. The committee must receive and review incident reports involving 1) exposures to biological agents or recombinant DNA, 2) theft or loss of biohazardous material, and 3) all incidents that warranted emergency response (See Sections 13.6(c)(4) and 13.11(a)-(c) for more on incident reports).

The IBC must report any noncompliance with IBC mandates/determinations, pertinent regulations, laws, guidelines, and University to the VPR. The committee must perform periodic reviews of this policy and suggest changes when needed to the VPR. This policy should be updated approximately every year.

Annually, in October, the IBC shall review the University's OSHA Exposure Control Plan.

13.3 Committee Composition

A. Appointment of Members

Voting members shall be appointed by the Chief Executive Officer in Consultation with the VPR. If authority to appoint is delegated, such delegation must be specific and in writing.

B Number of Members

The IBC shall consist of at least 5 members

C. Required Member Qualifications

The committee must collectively have experience and expertise in recombinant DNA technology and the capability to assess the safety of recombinant DNA research and to identify any potential risk to public health and/or the environment. The following are specific IBC membership requirements:

1. The University's Biosafety Officer shall be a member of the IBC (See Section 1.4.5 of this Policy for other Biosafety Officer responsibilities).
2. At least two members not affiliated with the University, besides membership on the IBC, must sit on the IBC to represent the community's health and environmental interests.
3. When submitted protocols involve research contained in Appendix P, *Physical and Biological Containment for Recombinant DNA Research Involving Plants*, a plant expert must sit on the committee to assist in conducting review.
4. When submitted protocols involve research contained in Appendix Q, *Physical and Biological Containment for Recombinant DNA Research Involving Animals*, an animal expert must sit on the committee to assist in conducting the review.

5. Whenever the University participates in or sponsors recombinant DNA research involving human subjects, the University shall ensure there is an IBC member who has sufficient experience and training in the field of human gene transfer.

6. When submitted protocols involve research outside the IBC's range of expertise, an Ad Hoc consultant shall be utilized.

7. It is recommended that at least one lab technical staff member sit on the IBC.

a. Ex Officio Members:

There shall be at least one member from the ORSP whose major role is research compliance appointed as an Ex Officio Member of the IBC.

E. OBA Contact Person

The Chair of the IBC shall serve as the contact person whom the OBA may contact with questions and important information regarding the University's IBC.

F. Rosters

Under Section IV-B-2-a-(3) of the *NIH Guidelines*, IBCs are required to submit committee rosters and bio-sketches of its members to the NIH. The NIH must disclose that information if requested to through the Federal Freedom of Information Act. See Section 12.4 (F)(2) below for redaction information.

13.4 Valid Method of IBC Review of Protocol Applications

Official business can only be validly conducted by a convened quorum of the IBC and members with sufficient expertise present. No process of expedited review in which one or more members reviews or approves protocols on behalf of the entire IBC is allowed. All members of the IBC shall have the opportunity to review all documents relating to a protocol at minimum 10 days prior to the convened meeting. IBC chair may assign one or more IBC members to conduct a specialized pre-review of a protocol prior to the meeting and present their findings at the convened meeting.

13.5 Meetings

A. Frequency

Meetings shall be held once a month unless there is no official business to conduct. At minimum, a meeting shall be held every six months.

B. Format

Meetings shall be in person or by tele/videoconference. E-mail is *not* an acceptable manner in which to conduct official business under any circumstances because e-mail does not allow for adequately preparing meeting minutes, nor accommodating public attendance.

C. Open to the Public

When possible and consistent with the protection of privacy and proprietary interests, the University will open its IBC meetings to the public. When meetings are open to the public, their time and place will be listed on the University's website. Any comments or questions received by the public regarding the committee's actions shall be responded to by the committee within 30 days. Any public comments regarding any action made by the IBC must be responded to and both the comments and IBC's response sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

D. Voting

1. Quorum Requirement for Conducting Official Business

A quorum is defined as greater than half of the voting members (at least 50% of the members plus 1). Official business shall only be conducted by a convened quorum. Actions (such as approval of a protocol) can only take place with a majority vote from a convened quorum.

2. Conflict of Interest

No member shall take place in the review or approval of a research protocol for which he or she has been or expects to be engaged in or have a direct financial interest. However, members with a conflict of interest may still provide information about the protocol to the IBC if necessary.

E. Attendance

Chronic nonattendance by members implies a lack of participation in oversight responsibilities of the IBC and often makes reviews of protocols impossible for lack of appropriate expertise. Chronic nonattendance will be addressed appropriately with such measures as adding additional members.

F. Minutes

1. Content/Detail

Minutes shall be taken every meeting and contain at minimum: the date and place of the meeting, whether minutes of the prior meeting were approved, individuals in attendance, whether and why the meeting was open or closed, all major motions, major points of order, and whether motions were approved, and the time of meeting adjournment. Minutes shall offer sufficient detail to serve as a record of major points of discussion and the IBC's rationale for particular decisions, documenting that the IBC has fulfilled its review and oversight responsibilities as outlined under Section IV-B-2-b of the *NIH Guidelines*. Minutes do not need to be transcripts or kept at a level of detail that attributes each remark to a specific individual.

2. Availability to the Public

Upon request, the University shall make meeting minutes available to the public. Requested minutes shall be distributed via mail or e-mail within 5 days of ORSP's receipt of the request. Pursuant to Section IV-B-2-1-(6) of the *NIH Guidelines*, the University may choose to redact information from meeting minutes and other documents that might become publicly available to protect private or proprietary information. Information that might be redacted, upon request from interested members, is trade secret information/other confidential commercial information, home telephone numbers and home addresses of IBC members, and specific information whose disclosure would directly compromise institutional or national security.

G. Principal Investigator Participation

PIs are encouraged to attend the meetings for which their protocols are being discussed. PIs shall leave the voting room or tele/videoconference before a vote is taken regarding their protocol.

13.6 IBC Review of Research Protocols

A. General

Before beginning research involving recombinant DNA or materials that are biosafety level 1 or higher, such protocols must be reviewed and approved by the IBC and the materials registered. Likewise, modifications and amendments to previously approved protocols must be reviewed. All protocols must be reviewed and re-approved annually to continue research. The IBC does not conduct expedited reviews. All official business must be conducted with a convened quorum to be in keeping with the *NIH Guidelines*.

B. Protocols Covered

All research and teaching protocols involving work with recombinant DNA and any materials that are Biosafety Level 1 or higher must be reviewed and approved before the study is commenced, and the materials registered with the IBC.

C. When/What Form(s) PIs Must Submit

1. Initial Application/Registration Document

To have the IBC review a protocol, the PI must submit an application form (See Appendix G of this Policy and document is available on IRBNet). If recombinant DNA is planned for use, the Principal Investigator shall also submit a rDNA Registration Form (See Appendix H of this Policy and document is available on IRBNet) specifying which section of the *NIH Guidelines* to which their research is subject, the source of the DNA, the host and vector to be used and containment conditions. Proof of completing training requirements (See Sections 1.4.1(J) and 13.8(1) of this Policy) and, when applicable, registration in the Occupational Health Program must be submitted with the application. Protocols will not be reviewed until such required proof is submitted.

a. Exemption.

Sections IV-B-2-b-(1) and 7-c-(3) of the *NIH Guidelines* cover recombinant work that is exempt from being registered. On the registration form, the Principal Investigator is asked to determine whether research is exempt under the *NIH Guidelines*,

however, the University requires all recombinant work to be registered with the IBC. The IBC Office and BSO in consultation with the IBC Chair verify whether such recombinant work determined exempt by the PI is in fact exempt for *NIH Purposes*. Even if such work is exempt for NIH purposes, the protocol will still be reviewed and subject to this policy if the material is biosafety level 1 or higher.

2. Annual Renewal of Protocol Approval and Registration

As part of the IBC's written approval of a PI's initial protocol and registration of materials, the IBC shall give notice to the PI of when he or she must submit an application for annual Re-Approval (Appendix G of this Policy and available on IRBNet). The PI will receive notice of upcoming approval expiration 60 days prior to the expiration date. The PI is responsible for submitting a continuing review application to the IBC 30 days prior to the project's expiration. When submitting the renewal application, keep in mind the 10 day rule described in Section 13.6(A) of this Policy. The IBC shall conduct de novo review of annual renewal applications.

3. Changes to Protocol

A Principal Investigator must submit a Protocol Amendment Form (Appendix G of this policy and available on IRBNet) if contemplating a change to a protocol. Such changes must be approved by the IBC *prior* to the changes being implemented.

4. Noncompliance Complaints/Adverse Events

Individuals aware of noncompliance with this policy or any of the policies, rules, regulations, and/or laws regarding research or teaching projects involving work with recombinant and/or other biohazardous materials should contact the Vice President for Research. Knowledge of the following events shall be submitted to the IBC for full committee review: 1) exposures to biological agents or recombinant DNA, 2) theft or loss of biohazardous material, and 3) all incidents that warranted emergency response. At that point the IBC shall conduct an investigation to determine whether the protocol must be modified or the research halted (See Section 14 of this Policy). Any of the above adverse events or those listed in Section 14 of this Policy must be

reported to the Vice President for Research Compliance. Both noncompliance complaints and adverse events must be submitted to the IBC with the appropriate information (See Sections 13.6(c)(4) and 13.11(a)-(c) of this Policy for guidance). In the event of an adverse event, see Section 11 of this policy and follow the instructions.

13.7 Timelines/Deadlines

A. Form Deadlines Prior to Review

Because all IBC members must have at least 10 days to review protocol documents before they are submitted, Principal Investigators must have all forms properly submitted, training completed, and be enrolled, when applicable, in the Occupational Health Program at least 10 days prior to the meeting in which they wish to have their protocol reviewed. Meeting dates and times are posted on the University's website once determined.

B. Protocol Approval Period Expiration

IBC approvals for protocols are valid for one year from the date specified as the approval date in the IBC's written approval document. There are absolutely no extensions or grace periods.

C. Consequences for Continuing Covered Research Activities After IBC Approval has Lapsed and Other Noncompliance

The Chief Executive Officer of the University has authorized the IBC to disapprove of or suspend research in noncompliance with policies and procedures described in the Biosafety Manual, NIH Guidelines and/or University policy, and the IBC has the responsibility to do so. Likewise, if a lab inspection results in a determination that basic lab procedures are not being followed, research may be halted until actions are taken to correct the deficiencies.

13.8 Review Criteria

A. The IBC shall review the following elements of covered protocols.

This list is not necessarily meant to be exhaustive:

1. Completion of training requirements.

As mandated in the *NIH Guidelines* Section IV-B-7-d-(2)-(3), the Principal Investigator is responsible for ensuring lab staff and others involved with research are sufficiently trained. The University requires compliance with its Training Program (Appendix A below). The PI is responsible for additional laboratory-specific training. The University shall inform the Principal Investigator of the training expectations and requirements under the *NIH Guidelines* by making available the OBA's instructional brochure (Appendix D of this Document). Proof that the appropriate personnel have satisfied training requirements outlined in Appendix A of this Policy is mandatory before a protocol is submitted to the IBC for review. Subsequently, the IBC can withhold approval if it deems additional training is necessary and has not been accounted for in the protocol. If the PI has administered lab-specific training, he or she must document that training as Specified in Section 1.4.1(J) of this Policy and submit a copy to the IBC and ORSP. See Section 1.4.1(J) for more on training and recordkeeping requirements related to training.

2. Compliance with University's Exposure Control Plan (Appendix C of this Policy), Occupational Health Program, and necessary vaccinations when applicable.
3. When work with animals is involved, verification that personnel are enrolled in Occupational Health Program as required by the University. IBC review shall be withheld at the administrative level until proof of enrollment in the OHP has been provided.

13.9 Outcomes of Protocol Review

A. There are three possible outcomes of review of an initial protocol application, annual renewal or modification:

1. Approval

Once the IBC renders approval, the IBC Office will send out an approval letter. After PI receipt of the letter, the approved study may be initiated. IBC approval is valid for one year.

2. Modifications Required to Secure Approval

The IBC may require modifications to the protocol before granting approval. In such cases, the Principal Investigator must modify the protocol and submit the modified protocol to

the IBC for full committee review. The IBC shall only grant approval after a convened quorum is satisfied that the required modifications have been made satisfactorily. The Principal Investigator shall not conduct research until all required modifications have been made and subsequently approved by the IBC. PIs must submit original protocol documents with the required modifications highlighted. Protocol Amendment Forms are inappropriate as they are only to be used for changes to previously approved protocols.

3. Approval Withheld

The IBC may withhold approval until the PI has made outlined modifications to the protocol. PI's are not to begin research until they have received an approval letter from the IBC Office.

B. The above actions require a formal vote of a convened IBC quorum.

C. Notification of Outcome

The IBC shall notify Principal Investigators and the University in writing of its decisions regarding protocols. If the IACUC withholds approval of an activity, it shall include in the written notification a statement of the reasons for its decision and give the Principal Investigator and opportunity to respond in person or in writing. A document indicating approval shall contain the date by which approval must be renewed.

13.10 Semi-Annual Review of Laboratories and Biosafety Program

A. At minimum every six months, the Biosafety Officer is responsible for conducting laboratory inspections to ensure that lab standards are being followed in accordance with federal/state/and local laws and guidelines. The Biosafety Officer shall report the results of all inspections to the IBC. If the Biosafety Officer or the IBC has reason to believe inspections should be carried out more frequently, the Biosafety Officer shall do so.

B. At minimum every year, in October, the IBC shall review the University's Bloodborne Pathogen Hazard Control Plan and general biosafety and IBC policies and procedures for compliance with current federal and state laws and regulations.

The IBC shall also conduct review of the biosafety program after significant adverse events in order to determine whether the event is a result of programmatic flaw.

13.11 Reporting

A. Internal Reporting

Annually the IBC shall submit a report to the Institutional Official (the VPR) to allow for assessment of the IBC's performance and compliance with the *NIH Guidelines*. The report shall include results of facility evaluations, an overview of the effectiveness of the biosafety program and bloodborne pathogens program and compliance with current federal regulations. Incidents listed in Section 13.11 B(3)(a) and 4 below shall be reported internally to the IBC and responsible official when they occur.

B. External Reporting

1. General/Reporting Responsibilities

When the University has an assurance with the OBA, the IBC is responsible for fulfilling reporting requirements to its office for covered research sponsored by or conducted by the University. The following outline specifics for reporting to the NIH OBA.

2. Annual Report

Annually the Institutional Official shall file an annual report with OBA in compliance with NIH-OBA requirements and procedures, which includes a roster of all IBC members and indicating the IBC chair.

3. Reports of Incidents, Accidents, or Violations to NIH and OBA

The *NIH Guidelines* require reporting of "any significant problems, violations of the *NIH Guidelines*, or any significant research-related accidents and illnesses" to the NIH and OBA within 30 days (*NIH Guidelines*, Section IV-B-2-b-(7)). Principal Investigators are responsible for reporting the following incidents to the Biosafety Officer and/or IBC. The Biosafety Officer is responsible for reporting any incidents reported to him or her to the IBC. The IBC is responsible for reporting incidents to the Institutional Official. The Institutional

Official is responsible for preparing and submitting the final incident report to the NIH OBA.

a. Events that must be reported to OBA include:

1. Any spill or accident involving recombinant DNA research as described in Section IV-B-2-b-(7) of the *NIH Guidelines*.
2. Any incident that leads to personal injury. An example includes skin punctures with needles containing recombinant DNA.
3. Any incident that leads to a breach of containment. Examples include the escape or improper disposition of a transgenic animal or spills of high-risk recombinant materials occurring outside of a biosafety cabinet.

Note: Minor spills of low-risk agents not involving a breach of containment that were properly cleaned and decontaminated generally do not need reported to the OBA. The OBA staff should be contacted when there is uncertainty surrounding whether or not to report and incident to their office.

4. Failure of a PI to adhere to the containment and biosafety practices articulated in the *NIH Guidelines*.
5. Events described in Appendix G of the *NIH Guidelines*. See Section 13.11(B)(3)(b) below.

b. Immediate Reporting

Certain events must be immediately reported to the NIH OBA:

1. Accidents as described in Appendix G of the *NIH Guidelines*. Examples include, spills or accidents in BSL-2 laboratories resulting in overt exposure (*NIH Guideline Appendix G-II-C-2-k*. Spills or accidents occurring in high containment (BSL-3 or BSL-4) resulting in an overt or potential exposure must be immediately reported to the IBC, BSO,

and OBA (*NIH Guideline* Appendices G-II-C-2-q and D-2-k).

c. Contents of Incident Reports

Incident reports must contain sufficient information to allow for an understanding of the nature, causes, and consequences of the incident. The report must also include the measures the University took to mitigate the problem and preclude its reoccurrence.

4. Adverse Events Involving Human Gene Transfer Trials

Adverse events in human gene transfer trials are subject to a separate set of reporting requirements outlined in the *NIH Guidelines*, Appendices M-1-C-3 and M-1-C-4. Serious and unexpected adverse events that are possibly associated with a gene transfer product must be reported to OBA within 15 calendar days of sponsor notification, unless they are fatal or life threatening, in which case they should be reported within 7 calendar days. Other serious adverse events should be reported to the OBA as part of the PI's annual report to the OBA

5. OBA Contact for Incident Reports

Incident Reports should be sent by mail to:

Dr. Kathryn Harris
National Institutes of Health
Office of Biotechnology Activities
6705 Rockledge Dr., Suite 750
Bethesda, MD 20892-7985

Dr. Kathryn Harris
National Institutes of Health
Office of Biotechnology Activities
6705 Rockledge Dr., Suite 750
Bethesda, MD 20817-1814

Fax: ATTN: Dr. Kathryn Harris
(301)496-9839

E-mail: HarrisKath@od.nih.gov

13.12 Recordkeeping

A. The University shall maintain records of the following IBC documents:

1. IBC minutes
2. Protocols reviewed and any attachments/amendments of protocol.
3. IBC member roster.

B. Length of Time Records Shall be Maintained:

1. All records shall be maintained for a period of at least three years. In the case of protocols and attachments/amendments, the three years begins three years after termination of the protocol.

C. Accessibility of Records

All records regarding covered projects shall be accessible for inspection and copying by authorized government representatives at reasonable times and in a reasonable manner.

14. Non-Compliance Complaints

14.1 Individuals concerned that there is non-compliance with this policy or any applicable policies, rules, regulations, and laws regarding biological safety in research and teaching, should contact the VPR.

14.2 Whistleblower Protection

Complaints are confidential and will remain anonymous to the largest extent possible. By law, such “whistleblowers” are protected from being retaliated/discriminated against. University policy further protects whistleblowers from retaliation.

14.3 Complaint/Investigation Procedure

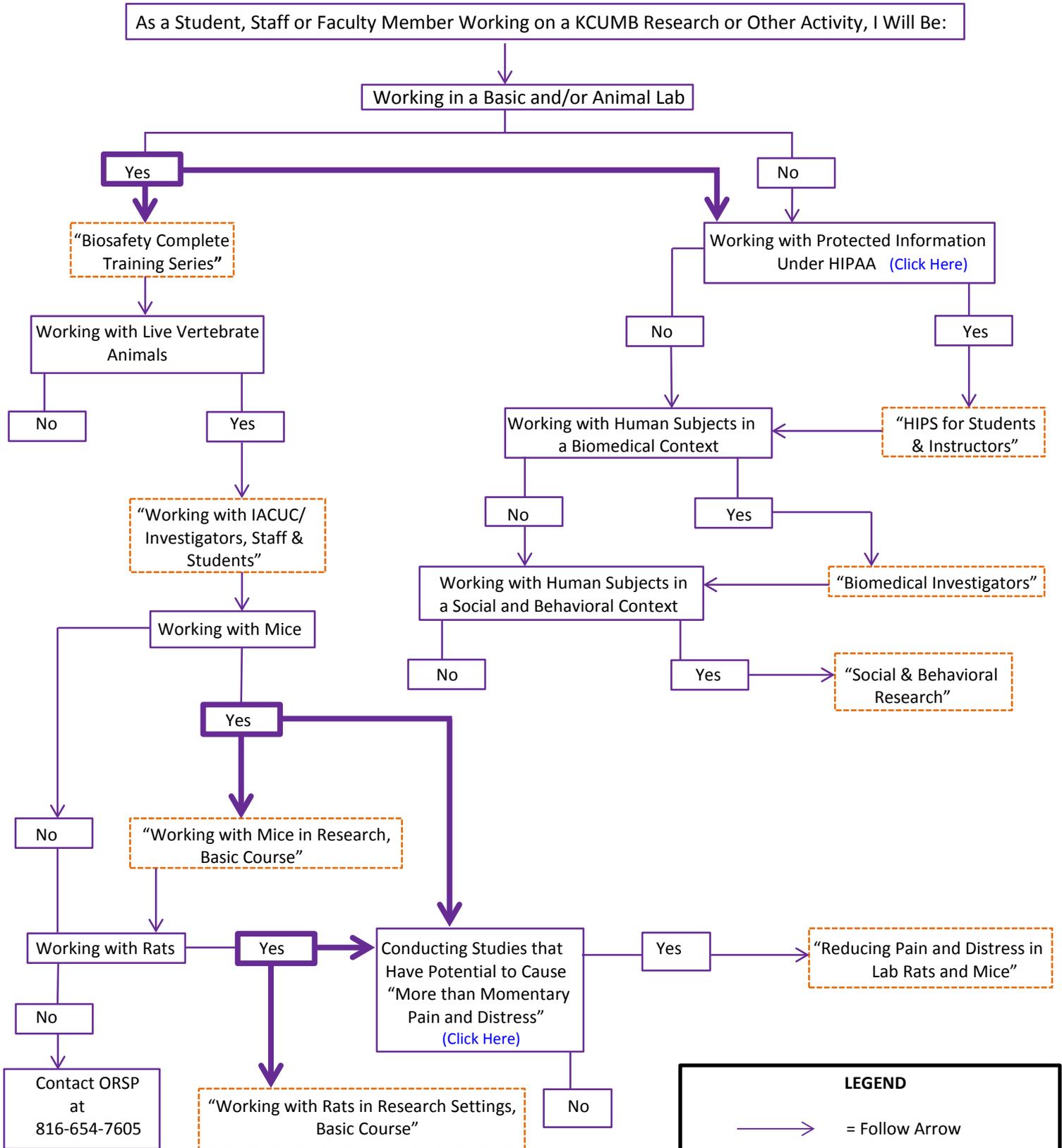
An individual reporting a complaint will remain anonymous to the extent reasonably possible if so requested by the individual. After receiving a complaint, the VPR will conduct an initial investigation to determine whether there is any credible evidence substantiating the claim. This initial investigation and an accompanying report shall be completed within 10 days from receipt of the complaint. Within that time period, if it is determined there is any credible evidence substantiating the claim, VPR shall draft his or her decision to conduct a further investigation in the aforementioned written report that includes what evidence has been found so far. Once a decision has been made to begin further investigation, the IBC, as well as any party who has been accused of culpability for the animal welfare violation, shall be notified if they have not already been so informed. Within 7 days from the determination that there is

evidence of a violation, the IBC chair shall appoint a subcommittee to further investigate the complaint. The subcommittee shall conduct a thorough investigation to be completed within 30 days of its appointment to gather information about the complaint. At the end of this 30 days, the full IBC shall be convened to hear the evidence surrounding the complaint, as well as witnesses and anyone against whom a complaint has been lodged, if such accused persons desire to be heard. After the meeting, the IBC shall decide if there has been animal welfare violation, and if so, what actions must be taken as well as a timeframe in which such actions should be completed. If there has been an animal welfare violation involving research funded by PHS, then as soon as possible, and no later than 10 days from the IBC's meeting surrounding the complaint, the committee must write a report as described in Section 14.3 of this policy, submit the report to the institutional official, and the institutional official shall submit the report to the appropriate PHS Official/Office as determined by usual methods of inquiry.

CITI TRAINING DECISION CHART

ACTIVITY(IES)	CITI COURSE(S) REQUIRED
Working with Protected Information Under HIPAA (Click Here)	“Health Information and Privacy (HIPS) for Students and Instructors”
Working with Human Subjects in a Biomedical Context	“Biomedical Investigators”
Working with Human Subjects in a Social & Behavioral Context	“Social & Behavioral Research”
Working in a Basic and/or Animal Laboratory	“Biosafety Complete Training Series”
Working with Live Vertebrate Animals	“Working with IACUC/Investigators, Staff & Students”
Working with Mice	“Working with Mice in Research, Basic Course”
Working with Rats	“Working with Rats in Research Settings, Basic Course”
Conducting Studies that Have Potential to Cause “More than Momentary Pain and Distress” (Click Here)	“Reducing Pain and Distress in Lab Rats and Mice”
Serving as Member on Institutional Review Board	“IRB Members”
Serving as Member on Biological Safety Committee	“Institutional Biosafety Committee Member”
Serving as Member on Animal Care and Use Committee	“Essentials for IACUC Members”

CITI TRAINING DECISION TREE



LEGEND

- = Follow Arrow
- = The CITI Course Needed
- = Two Arrows to Follow
- = Follow Both Bold Arrows

**NATIONAL INSTITUTES OF HEALTH
LABORATORY SAFETY MONOGRAPH**

*A SUPPLEMENT
TO THE
NIH GUIDELINES FOR RECOMBINANT DNA RESEARCH*

July 1978

LABORATORY SAFETY MONOGRAPH

A SUPPLEMENT TO THE NIH GUIDELINES FOR RECOMBINANT DNA RESEARCH

Prepared by the
Office of Research Safety
National Cancer Institute
and the
Special Committee of Safety and Health Experts

National Institutes of Health
Bethesda, Maryland

July 1978

U.S. Department of Health, Education, and Welfare
Public Health Service
National Institutes of Health

SPECIAL COMMITTEE OF SAFETY AND HEALTH EXPERTS

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Preface

The "Laboratory Safety Monograph—A Supplement to the NIH Guidelines for Recombinant DNA Research" is a revised and expanded edition of "Appendix D, Supplementary Information on Physical Containment," which was published June 23, 1976, as a part of the "NIH Guidelines for Recombinant DNA Research." This monograph was prepared in response to numerous requests for greater specificity in describing practices, equipment, and facilities appropriate for the safe conduct of recombinant DNA research.

The principal purpose of the "Laboratory Safety Monograph" is to assist scientific institutions, principal investigators, and health and safety professionals in the selection and use of physical containment measures described in the revised "NIH Guidelines for Recombinant DNA Research." The information provided in this monograph is based on established principles of laboratory safety, expert opinion, and experience in dealing safely with infectious disease organisms in diagnostic and research laboratories. The monograph will be useful, therefore, not only to those associated with recombinant DNA research, but to all who are associated with research programs involving potentially hazardous organisms.


Donald S. Fredrickson
Director, NIH

LABORATORY SAFETY MONOGRAPH
A SUPPLEMENT TO THE NIH GUIDELINES FOR RECOMBINANT DNA RESEARCH

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I. Introduction

The scientific community has long recognized the need to employ physical containment measures when conducting research with biological materials. Historically, the development and use of containment techniques began as an effort to secure and maintain pure cultures of bacteria; it was not until human laboratory-acquired infections began to appear that attention was given to the protection of personnel. For example, the frequency of typhoid among laboratory workers at the turn of the century caused by pipetting accidents, stimulated the development of mechanical pipetting aids. Since these early beginnings, a significant body of information has been developed by scientists and safety professionals, alike, which can be used today to guide laboratory workers in the safe conduct of research with potentially hazardous organisms. It is this body of information on which the "Laboratory Safety Monograph, a Supplement to the NIH Guidelines for Recombinant DNA Research" is based.

The "Laboratory Safety Monograph" provides information on physical containment measures that are applicable to recombinant DNA research. The monograph has been organized to complement the "NIH Guidelines for Recombinant DNA Research." Major sections of the monograph deal with laboratory practices, containment equipment, special laboratory design, and roles and responsibilities. The section on laboratory practices has information on specific techniques used by the laboratory worker in the control of biohazards. The section on containment equipment reviews Biological Safety Cabinets and describes their capabilities and limitations. This section also provides definitive procedures for certifying the containment capability of Biological Safety Cabinets. The special laboratory design section amplifies design considerations for P3 and P4 facilities; certification procedures for important facility safeguards are presented in this section. The section on roles and responsibilities emphasizes the activities of the Institutional Biosafety Committee and the biological safety officer. Guidance concerning emergency procedures and medical surveillance is provided. This section also

identifies specific training resources and provides a reference bibliography on biological safety. The monograph also includes guidelines for the control of moderate risk oncogenic viruses and information on packaging and shipping of recombinant DNA materials.

Experience has demonstrated that the safe conduct of research involving potentially hazardous organisms is dependent on good laboratory practice, the availability and use of containment equipment, the design and operation of the research facility, and effective management. These are the subject areas to which this monograph has been addressed. It is hoped that this information will be of value to all concerned with the safe conduct of recombinant DNA research.

Finally, the suggestions and recommendations presented in the monograph are advisory in nature; they do not constitute mandatory requirements. An important intent of the monograph is to encourage investigators, safety professionals and institutional officials to seek new and better methods of biohazard control and to apply professional judgement in the interpretation of the physical containment requirements of the "NIH Guidelines for Recombinant DNA Research."

Note: This monograph names and illustrates representative materials, processes, and equipment only, and should not be considered as an all-inclusive listing. Names of commercial manufacturers and trade names are provided as examples only, and their inclusion does not constitute or imply approval or endorsement; nor does the exclusion of commercial manufacturers' names and trade names constitute or imply nonapproval or nonendorsement by the National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

II. Laboratory Practices

A. Laboratory Techniques for Biohazard Control

Analysis of comprehensive surveys of laboratory-acquired infections (1)* indicates that fewer than 20 percent of known infections can be attributed to a documented accidental exposure. Risk assessment studies, however, have demonstrated that aerosols are created by most laboratory manipulations that involve microorganisms (2-5). These results suggest that inhalation of undetected aerosols may have contributed to occupational illness among laboratory workers who have handled biohazardous materials (6,7).

Laboratory techniques for operations that have a high potential for creating aerosols are reviewed in this section. Four measures are recommended to decrease the hazard of exposure to aerosols.

MEASURES TO DECREASE HAZARDS OF AEROSOLS FROM LABORATORY OPERATIONS

Operations that have the potential to create hazardous aerosols	Measures to decrease hazards from aerosols:			
	Avoid creating aerosol	Reduce extent of aerosol	Contain aerosol	Use personal respiratory protection
blowing the last drop of a liquid culture or chemical from a pipette	●			
removing the cover from a Waring blender or dry chemical grinder shortly after completion of the blending or grinding operation		●	●	
removing the cap from a bottle of a liquid culture or suspension immediately after vigorous shaking; improper stoppering of volatile toxic substances		●	●	
grinding tissue with mortar and pestle or glass tissue grinder		●	●	
decanting the supernatant fluid after centrifugation	●	●		
resuspending packed cells by shaking or mixing		●	●	
inserting a hot wire loop in a culture	●	●		
withdrawing a culture sample from a vaccine bottle	●	●		
opening a freeze-dried preparation		●	●	
shaking and blending cultures and infected tissues in high-speed mixers		●	●	
disrupting tissue cultures to release virus by shaking with glass beads		●	●	
streaking an inoculum on a rough agar surface	●			
sonic disruption of cells		●	●	
inoculating mice via the intranasal or other routes		●	●	
harvesting cultures from embryonated eggs	●	●	●	
evacuating the atmosphere from a high-vacuum steam sterilizer prior to sterilization of contaminated material	●	●		
removing cotton plugs from flasks and centrifuge tubes		●		
handling cages that held infected animals		●	●	●
handling large animals in open areas or in unventilated cages		●		●

*The references for this section appear on page 39.

Measures that avoid the creation of an aerosol or reduce the extent of aerosol formation should be employed routinely. Measures that contain the aerosol are to be used when the research activity requires physical containment at levels of P2 and above.

1. Pipetting

Pipettes are basic scientific pieces of equipment used throughout the world. They are used for volumetric measurement of fluids and for the transfer of these fluids from one container to another. The fluids that are handled are frequently hazardous in nature, containing infectious, toxic, corrosive or radioactive agents. A pipette can become a hazardous piece of equipment if improperly used. Safety pipetting techniques are required to reduce the potential for exposure to hazardous materials. The most common hazards associated with pipetting procedures involve the application of mouth suction. The causative event in more than 13 percent of all known laboratory accidents that resulted in infection was oral aspiration through a pipette. Contaminants can be transferred to the mouth if a contaminated finger is placed on the suction end of the pipette. There is also the danger of inhaling aerosols created in the handling of liquid suspensions when using unplugged pipettes, even if no liquid is drawn into the mouth. Additional hazards of exposure to aerosols are created by liquid dropping from a pipette to a work surface, by mixing cultures by alternate suction and blowing, by forceful ejection of an inoculum onto a culture dish, or by blowing out the last drop. It has been demonstrated by high-speed photography that an aerosol of approximately 15,000 droplets, most under ten micrometers, is produced when the last drop of fluid in the tip of the pipette is blown out with moderate force. While the aerosol hazard associated with pipetting procedures can only be reduced by use of safe techniques and of Biological Safety Cabinets, the potential hazards associated with oral ingestion can be eliminated by use of mechanical pipetting aids.

a. Safety Pipetting Aids

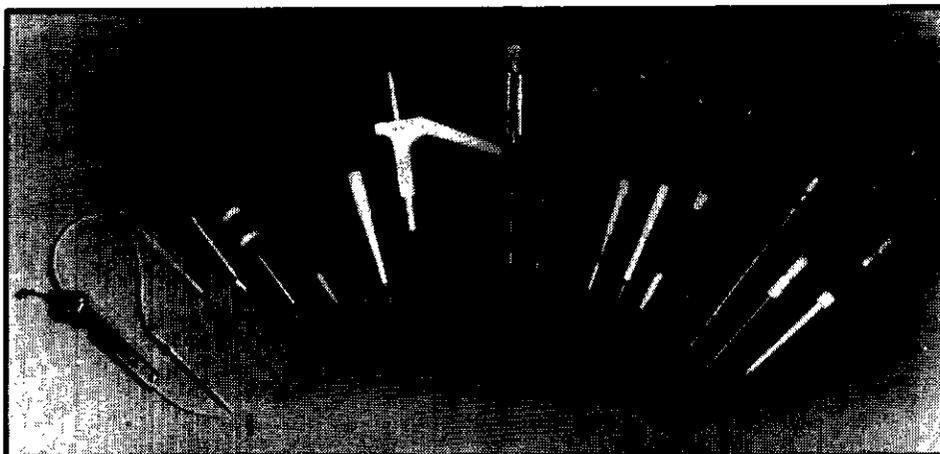
There are many commercially available safety pipetting aids that can be used. A particular type of pipetting aid that may be satisfactory to one individual or kind of operation may not meet the requirements of others; therefore, different types should be tried in each

situation. Ease of manipulation and accuracy of delivery by the aids are important factors in their selection. Care should be taken in selecting a device that its design or use does not contribute to exposure to hazardous contaminants, that exposed parts (other than delivery tips) are kept free of hazardous substances, and that the unit can be easily sterilized and cleaned after use. Any device requiring mouth suction should be considered unsafe.

For research activities, there is no standard safety pipetting aid; instead, there is a wide choice of hand-held, nonautomatic devices to meet different needs. Nonautomatic pipetting aids are available in a variety of bulb-actuated suction devices and piston or syringe types. Some may be designed to provide easy repetitive withdrawal of aliquots of a fluid. Many of those that are in use are illustrated below. Others are available and new ones are developed and put on the market each year. Detailed information on these products can be obtained by writing to the manufacturer.

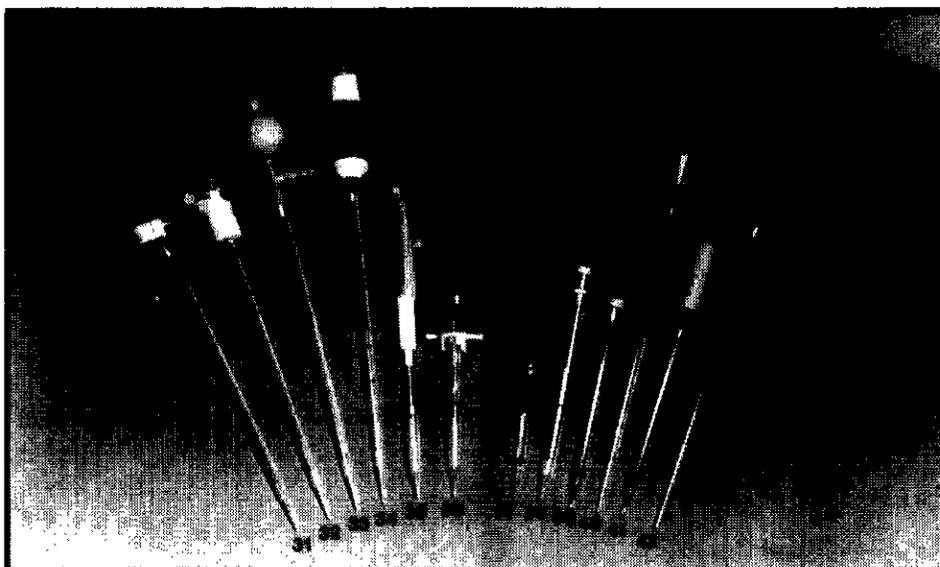
The pipetting aids used in biomedical and chemical laboratories can be classified into three categories: ultramicro (<0.1 ml); micro (0.1 ml to 1 ml), and macro (>1 ml). Some systems use disposable tips and some a small vacuum pump. The latter incorporates a separate high-efficiency particulate air (HEPA) filter system intended to prevent the escape of any potential hazardous aerosol; the nosepiece contains a "fail safe" check valve for preventing the sample from being accidentally drawn into the handle or pump. In selecting a pipetting aid, consideration should be given to whether the unit can be sterilized with steam and to the ease of reading the meniscus, controlling the flow of fluid, filling, discharging, and changing pipettes. Other desirable features of design are that it not contribute to fatigue of the finger and wrist, that it leaves the third and fourth fingers free to manipulate plugs and bottle caps, and that it not contribute to lack of control and cause leaking.

Sources and prices (1977) are given in the table following the illustrations.



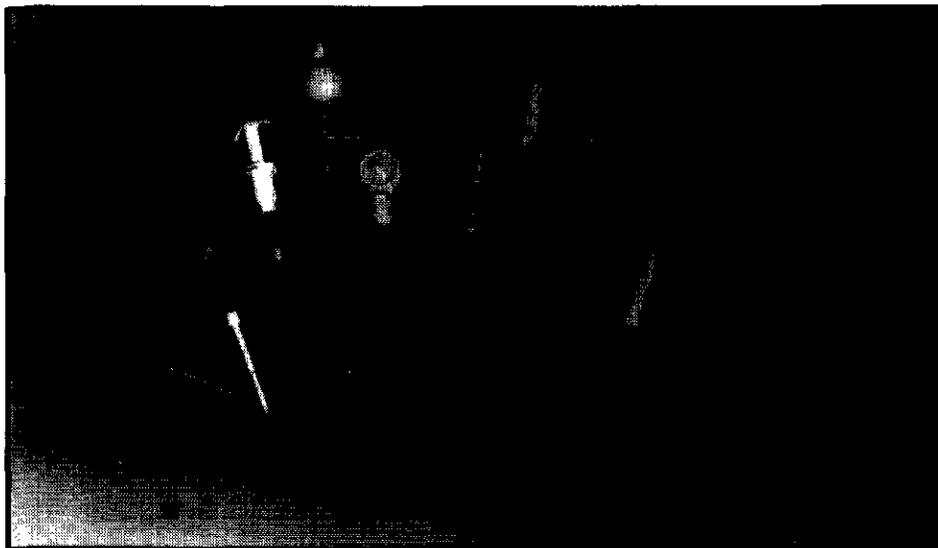
- | | | |
|---------------------------------------|--|---|
| 1 Clinac Safety Pipettor | 8 Clay Adams-Micro Selectapette | 14 Oxford Sampler Ultramicropipette |
| 2 Sargent-Welch Pipette Syringe | 9 Lancer Precision Pipettor | 15 Elkay "Socorex" Micro Pipette (Swiss manufactured) |
| 3 Drummond Dialomatic Micro Dispenser | 10 MLA Pipettor | 16 Centaur Pipet |
| 4 Hamilton Ultra Micro Syringe Pipet | 11 Eppendorf Microliter Pipet (West German manufactured) | 17 Oxford Sampler Pipette, Model Q |
| 5 Unimetrics Micropipettor | 12 Pipetman Ultra Micro Pipette (French manufactured) | 18 Gilson/Rainin Pipetman, Digital Dispensing Pipette (French manufactured) |
| 6 Finnpiquette (Finnish manufactured) | 13 Oxford Sampler Pipette (8000 Series) | |
| 7 Helena Quickpette | | |

ULTRAMICRO PIPETTING AIDS



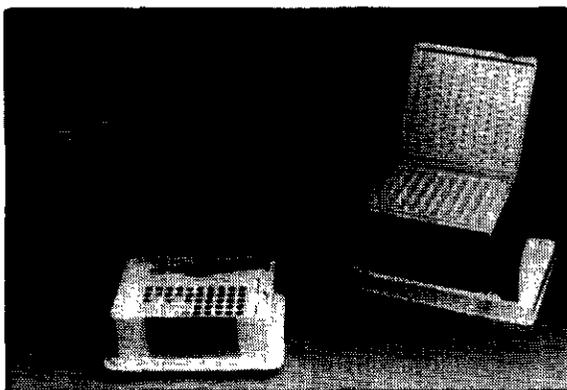
- | | |
|--|--|
| 31 National Instrument Micropipet Pipettor (French manufactured) | 37 Labindustries Micro/Mac Pipet |
| 32 Pumpett 25, Original (Swedish manufactured) | 38 Centaur |
| 33 Spectroline Pipette Filler | 39 Interex Rubber Transfer Bulb |
| 34 Manostat Accropet Filler (Macro) | 40 Volac Universal Pipette Controller (British manufactured) |
| 35 Oxford Macro Set Transfer Pipetting System | 41 Nalgene Pipetting Aid |
| 36 Lab Industries Repipet Jr Sampler | 42 Pi Pump (West German manufactured) |

MICRO PIPETTING AIDS

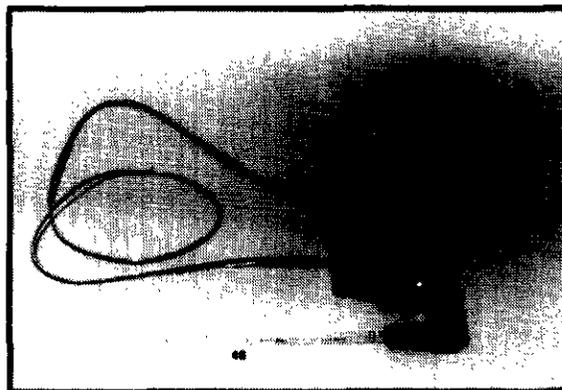


- | | | |
|---|---|--|
| 19 Biopipette Automatic Pipette | 23 Pumpett 18
(Swedish manufactured) | 27 Analytic Products Safety Bulb |
| 20 Gilson/Rainin Pipetman, Digital
Dispensing Pipette
(French manufactured) | 24 Curtin Matheson Rubber Pipet Bulb | 28 Clay Adams Pipet Suction
Apparatus |
| 21 Manostat Vari-Pet | 25 Demuth Safety Pipet | 29 Manostat Accropet Filler |
| 22 Spectroline Pipette Filler | 26 Nalgene Pipetting Aid | 30 Cornwall Continuous Pipetting
Outfit |

MACRO PIPETTING AIDS



- 43 MLA Inc. Pipetting System
- 44 Clay Adams Selectapette Pipet System
- 45 Centaur Pipet System



- 46 Drummond Pipet-Aid

PIPETTING AID SYSTEMS

SOURCES OF PIPETTING AIDS

Item / Source	Figure Legend Numbers	Price Range*	Item / Source	Figure Legend Numbers	Price Range*
Analytic Products Safety Bulb Analytical Products, Inc. P.O. Box 845 Belmont, CA 94002	27	C	Drummond Pipet-Aid	46	J
Biopipette Automatic Pipette Schwartz/Mann Division of Becton, Dickinson & Co. Orangeburg, NY 10962	19	I	Elkay "Socorex" Micro Pipette (Swiss manufactured) Elkay Products, Inc. 95 Grand Street Worcester, MA 01610	15	H
Centaur Pipet Centaur Chemical Company 180 Harvard Avenue Stanford, CT 06902	16, 38	G, H	Eppendorf Microliter Pipet (West German manufactured) Brinkman Instruments, Inc. Cautrague Road Westbury, NY 11590	11	H
Centaur Pipet System	45	H	Finnpipette (Finnish manufactured) Variable Volumetrics, Inc. 17 Cummings Park Woburn, MA 01801	6	I
Clay Adams-Micro Selectapette Clay Adams Division of Becton, Dickinson & Co. Parsippany, NJ 07054	8	I	Gifson/Rainin Pipetman, Digital Dispensing Pipette (French manufactured) Rainin Instrument Company, Inc. 94 Lincoln Street Brighton, MA 02135	18, 20	I
Clay Adams Pipet Suction Apparatus	28	C	Hamilton Ultra Micro Syringe Pipet Hamilton Company P.O. Box 7500 Reno, NV 89502	4	H
Clay Adams Selectapette Pipet System	44	I	Helena Quickpette Helena Laboratories P.O. Box 752 Beaumont, TX 77704	7	H
Clinac Safety Pipettor LaPine Scientific Company 6001 South Knox Avenue Chicago, IL 60629	1	E	Interex Rubber Transfer Bulb Interex Corporation 3 Strathmore Road Natick, MA 01760	39	A
Cornwall Continuous Pipetting Outfit Curtin Matheson Scientific Co. P.O. Box 1546 Houston, Texas 77001	30	E	Labindustries Micro/Mac Pipet Labindustries 1802 Second Street Berkeley, CA 94710	37	B (glass) A (rubber)
Curtin Matheson Rubber Pipet Bulb Curtin Matheson Scientific Co. P.O. Box 1546 Houston, Texas 77001	24	A	Labindustries Repipet Sampler	36	E
Demuth Safety Pipet Demuth Glass Division Brockway Glass Company, Inc. Route 1, Box 13 Parkersburg, WV 26101	25	A	Lancer Precision Pipettor Sherwood Medical Industries 1831 Olive Street St. Louis, MO 63103	9	H
Drummond Dialomatic Micro Dispenser Drummond Scientific Company 500 Parkway Broomall, PA 19008	3	H			

SOURCES OF PIPETTING AIDS (Continued)

Item / Source	Figure Legend Numbers	Price Range*	Item / Source	Figure Legend Numbers	Price Range*
Manostat Accropet Filler Manostat Corporation 519 Eighth Avenue New York, NY 10018	29, 34	C	Pi Pump (West German manufactured) Bel Art Products Pequannock, NJ 07440	42	D
Manostat Vari-Pet	21	G	Pipetman Ultra Micro Pipette (French manufactured) Analtech, Inc. 75 Blue Hen Drive Newark, DE 19711	12	I
MLA Pipettor & Pipettor System Medical Laboratory Automation, Inc. 520 Nuber Avenue Mount Vernon, NY 10550	10, 43	H, I	Pumpett 18 (Swedish manufactured) LaPine Scientific Company 6001 South Knox Avenue Chicago, IL 60629	23	C
Nalgene Pipetting Aid (West German manufactured) Nalgene Labware Division Nalge Sybron Corporation P.O. Box 365 Rochester, NY 14602	26, 41	D	Pumpett 25, Original (Swedish manufactured)	32	C
National Instrument Micropipet Pipettor (French manufactured) National Instrument Co., Inc. 4119 Fordleigh Road Baltimore, MD 21215	31	D	Sargent-Welch Pipette Syringe Sargent-Welch Scientific Co. 7300 North Linder Avenue Skokie, IL 60076	2	D
Oxford Macro Set Transfer Pipetting System Oxford Laboratories 1149 Chess Drive Foster City, CA 94404	35	I	Spectroline Pipette Filler Arthur H. Thomas Company Vine Street at 3rd P.O. Box 779 Philadelphia, PA 19105	33, 22	D
Oxford Sampler Pipette (8000 Series)	13	H	Unimetrics Micro-pipettor Unimetrics Corporation 1853 Raymond Avenue Anaheim, CA 92801	5	I
Oxford Sampler Pipette, Model Q	17	H	Volac Universal Pipette Controller (British manufactured) Cole-Parmer Instrument Company 7425 North Oak Park Avenue Chicago, IL 60648	40	D
Oxford Sampler Ultramicropipette	14	H			

***PRICE RANGES**

A \$ 5.00 or less	E \$10.01 - \$25.00	H \$ 30.01 - \$ 50.00
B \$ 1.00 - \$10.00	F \$20.01 - \$30.00	I \$ 50.01 - \$100.00
C \$ 5.01 - \$10.00	G \$25.01 - \$50.00	J \$100.00 or more
D \$10.01 - \$20.00		

The older conventional transfer and serological pipettes are difficult to use in safety cabinets because of their length. In these instances, select an appropriate short pipette from those now commercially available.

When it is necessary to insert the suction end of the pipette into the pipetting aid, this can be done more easily if the suction end is first wetted by a small sponge soaked in a disinfectant. This will also assist in achieving an airtight closure.

Prospective users of pipetting aids must be cautioned that no independent studies have been reported on the quantitative assessment of the safety performance of the aids. It is desirable that such studies be undertaken within each laboratory group for its particular operation. The parameters that deserve attention in a safety evaluation involve the ability of the pipetting aid:

- To perform without uncontrollable discharge (i.e., leakage) from the pipette or disposable tip.
- To function without contamination of the suction end of the pipette and, in turn, the pipetting aid, operator and vacuum lines.
- To transfer fluids without creating aerosols that spread surface contamination and result in inhalation and ingestion of hazardous substances.
- To be cleaned and sterilized in routine maintenance and preventive operations or following overt accidental contamination.

b. Safe practices governing the use of pipettes and pipetting aids.

(1) Never use mouth pipetting. Always use some type of pipetting aid.

(2) If working with biohazardous or toxic fluids, pipetting operations should be confined to a safety cabinet or hood.

(3) Pipettes used for the pipetting of biohazardous or toxic materials always should be plugged with cotton (even when safety pipetting aids are used).

(4) No biohazardous material should be prepared by bubbling expiratory air through a liquid with a pipette.

(5) Biohazardous material should not be mixed by suction and expulsion through a pipette.

(6) No biohazardous material should be forcibly expelled out of a pipette.

(7) When pipettes are used, avoid accidentally dropping infectious cultures from the pipette. Place a disinfectant-soaked towel on the working surface and autoclave the towel after use.

(8) Mark-to-mark pipettes are preferable to other types, since they do not require expulsion of the last drop.

(9) Discharge from pipettes should be as close as possible to the fluid or agar level, or the contents should be allowed to run down the wall of the tube or bottle whenever possible, not dropped from a height.

(10) Contaminated pipettes should be placed horizontally in a pan containing enough suitable disinfectant to allow complete immersion. They should not be placed vertically in a cylinder.

(11) Discard pans for used pipettes are to be housed within the Biological Safety Cabinet.

(12) The pan and pipettes should be autoclaved as a unit. The replacement unit should be a clean pan with fresh disinfectant.

2. Syringes and Needles (8)

The hypodermic needle is a dangerous instrument. To lessen the chance of accidental injection, aerosol production or spills, its use should be avoided when alternate methods are available. For example, use a blunt needle or a cannula on the syringe for oral or intranasal inoculations and never use a syringe and needle as a substitute for a pipette in making dilutions of dangerous fluids.

The following practices are recommended for use of the hypodermic needle and syringe when used for parenteral injections:

- Use the syringe and needle in a Biological Safety Cabinet only and avoid quick and unnecessary movements of the hand holding the syringe.
- Examine glass syringes for chips and cracks, and needles for barbs and plugs. This should be done prior to sterilization before use.
- Use needle-locking (Luer-Lok^R type) syringes only, and be sure that the needle is locked securely into the barrel. A disposable syringe-needle unit (where the needle is an integral part of the unit) is preferred.
- Wear surgical or other type rubber gloves for all manipulations with needles and syringes.
- Fill the syringe carefully to minimize air bubbles and frothing of the inoculum.
- Expel excess air, liquid and bubbles from a syringe vertically into a cotton pledget moistened with the proper disinfectant, or into a small bottle of sterile cotton.
- Do not use the syringe to expel forcefully a stream of infectious fluid into an open vial or tube for the purpose of mixing. Mixing with a syringe is condoned only if the tip of the needle is held below the surface of the fluid in the tube.
- If syringes are filled from test tubes, take care not to contaminate the hub of the needle, as this may result in transfer of infectious material to the fingers.
- When removing a syringe and needle from a rubber-stoppered bottle, wrap the needle and stopper in a cotton pledget moistened with the proper disinfectant. If there is danger of the disinfectant contaminating sensitive experimental materials, a sterile dry pledget may be used and discarded immediately into disinfectant solution.

- Inoculate animals with the hand "behind" the needle to avoid punctures.
- Be sure the animal is properly restrained prior to the inoculation, and be on the alert for any unexpected movements of the animal.
- Before and after injection of an animal, swab the site of injection with a disinfectant.
- Discard syringes into a pan of disinfectant without removing the needle or manually replacing the protective needle sheath that is furnished with disposable hypodermic needles or syringe-needle units. The syringe may be filled with disinfectant by immersing the needle and slowly withdrawing the plunger, and finally removing the plunger and placing it separately into the disinfectant. The filling action clears the needle and dilutes the contents of the syringe. Autoclave syringes and needles in the pan of disinfectant.
- In instances where the protective needle sheath must be replaced following use of a syringe (e.g., blood samples drawn for diagnostic purposes), forceps should always be used to minimize the possibility of exposure via accidental autoinoculation.
- Use separate pans of disinfectant for disposable and nondisposable syringes and needles to eliminate a sorting problem in the service area.
- Do not discard syringes and needles into pans containing pipettes or other glassware that must be sorted out from the syringes and needles.

3. Freeze-Drying Specimens

Specimens shell-frozen in ampoules, are dried on a vacuum manifold or in a chamber-type drier at low negative pressure. If the glass neck of the ampoule is sealed off while it is still under vacuum, it may cause implosion either during sealing or later when the evacuated ampoule is being opened. To avoid this, after drying is completed and before sealing is done bring the pressure within the ampoule back to normal by gradually introducing dry nitrogen, avoiding turbulent disturbance of the dry product.

The narrow or constricted neck of ampoules is contaminated if the specimen is allowed to run down the wall of the neck during filling. Subsequently, when the ampoule is sealed with a torch, the dried material on the wall becomes charred or partially decomposed; residues of this material may adversely affect the dried material when it is reconstituted. To avoid this, a syringe with a long cannula or a Pasteur-type pipette should be used to fill the vial. Do not allow the delivery end of the cannula or pipette to touch the neck of the vial.

All ampoules used for freeze-drying of cultures, toxins or other hazardous materials should be fabricated in Pyrex-type glass. This glass requires a high-temperature torch using an air-gas or oxygen-gas mixture for sealing. These hard glass ampoules are much less apt to form glass bubbles that burst inwardly during sealing under vacuum than the soft glass ampoules and, of course, are more resistant to breakage from heat-shock, handling and storage.

The freeze-drier tubes, manifold, condenser pump and other internal parts will be contaminated after use (9). When dry infectious organisms are being prepared, a significant biohazard exists. Whether infectious or noninfectious living organisms, or toxic or nontoxic nonliving agents are being treated, if purity of product is a major concern, decontamination should be carefully considered. Protection by use of air filters should be given to vacuum lines and oil pumps. Freon-ethylene oxide gas, steam sterilizing, and liquid decontaminants should be employed as

appropriate, depending on the biological or chemical agent in use and the composition of mechanical parts of the drier.

The changer-type freeze-driers allow the use of automatic plugging and screw-capping of vials and also the use of tray-drying in place of drying in ampoules or vials. The biohazards and chances of product contamination still exist and must be considered as with manifold-drying systems.

Protection against contamination of vacuum lines and pumps should be provided by appropriate filter systems.

The filling of ampoules and vials with infectious specimens and subsequent freeze-drying and sealing or closing of glass ampoules and vials in the preparation of dry infectious specimens should be done in a Biological Safety Cabinet. The same is true for preparation of ampoules and vials of liquid specimens not subjected to freeze-drying.

4. Opening Culture Plates, Tubes, Bottles, and Ampoules; Inoculating and Harvesting Cultures.

In the absence of definite accidents or obvious spillage, it is not certain that opening of plates, tubes and bottles of other microorganisms has caused laboratory infection. However, it is probable that among the highly infective agents some infections have occurred by this means (9). Particular care is required when opening plates, tubes, or bottles containing fungi, for this operation may release a large number of spores. Such cultures should be manipulated in a Biological Safety Cabinet (10,11).

To assure a homogenous suspension that will provide a representative sample, liquid cultures are agitated before a sample is taken. Vigorous shaking will create a heavy aerosol. A swirling action will generally create a homogenous suspension with a minimum of aerosol. When a liquid culture is resuspended, a few minutes should elapse prior to opening the container to reduce the aerosol.

The insertion of a sterile, hot wire loop or needle into a liquid or slant culture can cause spattering and release of an aerosol. To minimize the aerosol production, the loop should be allowed to cool in the air or be cooled by touching it to the inside of the container or to the agar surface where no growth is evident prior to contact with the culture or colony. Following use of the inoculating loop or needle, it is preferable to sterilize the instrument in an electric or gas incinerator specifically designed for this purpose rather than heating in an open flame. These small incinerators have a shield to contain any material that may spatter from the loop. Disposable inoculating loops are available commercially. Rather than decontaminating them immediately after use with heat, they are discarded first into a disinfectant solution.

The practice of streaking an inoculum on rough agar results in aerosol production, created by the vibrating loop or needle. This generally does not occur if the operation is performed on smooth agar. It is good safety practice to discard all rough agar poured plates that are intended for streaking purposes with a wire loop.

Water of syneresis in petri dish cultures usually contains viable microorganisms and forms a film between the rim and lid of the inverted plate. Aerosols are dispersed when this film is broken by opening the plate. Vented plastic petri dishes where the lid touches the rim at only three points are less likely to offer this hazard (12). The risk may also be minimized by using properly dried plates, but even these (when incubated anaerobically) are likely to be wet after removal from an anaerobic jar. Filter papers fitted into the lids reduce, but do not prevent, dispersal. If plates are obviously wet, they should be opened in the Biological Safety Cabinet (13).

Less obvious is the release of aerosols when screw-capped bottles or plugged tubes are opened. This happens when a film of contaminated liquid, which may collect between the rim and the liner, is broken during removal of the closure (6). The practice of removing cotton plugs or other closures from flasks, bottles, centrifuge tubes, etc., immediately following shaking or centrifugation can generate aerosols and cause environmental contamination. The technique of shaking tissue cultures with glass beads to release viruses can create a virus-laden aerosol. Removal of wet closures, which can occur if the flask or centrifuge tube is not held in an upright position, is also hazardous. In addition, when using the centrifuge, there may be a small amount of foaming and the closures may become slightly moistened. Because of these possibilities, it is good safety practice to open all liquid cultures of infectious or hazardous material in a Biological Safety Cabinet wearing gloves and a long-sleeved laboratory garment.

Dried, infectious culture material may also collect at or near the rim or neck of culture tubes and may be dispersed into the air when disturbed (14). Containers of dry powdered hazardous materials should be opened only in a Biological Safety Cabinet (10,15).

When a sealed ampoule containing a lyophilized or liquid culture is opened, an aerosol may be created. Aerosol creation should be prevented or minimized, and opening of ampoules should be done in safety cabinets.

When recovering the contents of an ampoule, care should be taken not to cut the gloves or hands or disperse broken glass into the eyes, face, or laboratory environment. In addition, the biological product itself should not be contaminated with foreign organisms or with disinfectants. To accomplish this—work in a safety cabinet and wear gloves. Nick the ampoule with a file near the neck. Wrap the ampoule in disinfectant-wetted cotton. Snap the ampoule open at the nick, being sure to hold the ampoule upright. Alternatively, at the file mark on the neck of the ampoule, apply a hot wire or rod to develop a crack. Then, wrap the ampoule in disinfectant-wetted cotton, and snap it open. Discard cotton and ampoule tip into disinfectant. The contents of the ampoule are reconstituted by slowly adding fluid to avoid aerosolizing the dried material. Mix contents without bubbling, and withdraw it into a fresh container (13). Some researchers may desire to use commercially available ampoules prescored for easy opening. However, there is the possibility to consider that this may weaken the ampoule and cause it to break during handling and storage. Ampoules of liquid cultures are opened in a similar way.

Harvesting cultures from embryonated eggs is a hazardous procedure and leads to heavy surface contamination of the egg trays, shells, the environment, and the hands of the operator. It is essential that operations of this type be conducted in a Biological Safety Cabinet. A suitable disinfectant should be at hand and used frequently.

5. Centrifuging

Centrifugation presents two serious hazards: mechanical failure and dispersion of aerosols. A mechanical failure, such as a broken drive shaft, a faulty bearing, or a disintegrated rotor, can produce not only aerosols but also hazardous fragments moving at great velocity. These fragments, if they escape the protective bowl of the centrifuge, could produce traumatic injury to personnel. A well-functioning centrifuge, however, is still capable of producing hazardous aerosols of biological material or chemicals if improperly used or in the absence of good laboratory practices. Mechanical failure can be minimized by meticulous observance of the manufacturers' instructions, and aerosols can be avoided by observing sound laboratory practices and use of appropriate centrifuge safety equipment or Biological Safety Cabinets (16-23).

Although accidents from improper use of centrifuges and equipment associated therewith are far less frequent than with pipettes or syringes and needles, when they do occur aerosols usually are created, and the possibility of causing multiple exposures is considerably greater. This conclusion is borne out by data presented in the following table from the proceedings of a symposium held on centrifuge biohazards in 1973 (24).

INFECTION OR HYPERSENSITIVITY FROM CENTRIFUGING MICROBIAL MATERIAL

Disease	Comment	Persons Affected
Brucellosis	Aerosol spread from basement to 3rd floor	94
Glanders	Tube broke	3 (2 fatal)
Plague	Fluid spun off lip of intact centrifuge tube	1
Q fever	"Use of a centrifuge"	60
Q fever	Spread from 1st to 3rd floor*	47
Q fever	Throughout the building, "Centrifuging or grinding tissue"	15
Tuberculosis	Broken tube and a hole in trunnion cup	2
Tularemia	"Principally the pipetting and centrifugation"	1
Tularemia	Centrifuging	1
Western equine encephalitis	"Virus was thrown out"	1
Allergy attacks	Preparing antigens in a Sharples centrifuge	7
Allergy attacks	Killed <u>M. tuberculosis</u>	1

*Waring blender used, and "high-speed centrifugation of formalinized suspensions and subsequent resuspension of sediments."

Activities, such as filling centrifuge tubes, removing cotton plugs and rubber caps from tubes after centrifugation, removing the supernatant and resuspending the cells, are capable of releasing aerosols into the environment. The greatest hazard associated with centrifuging biohazardous materials is created when a centrifuge tube breaks. When tubes break or crack and a fluid containing microorganisms remains in the cup under centrifugal force, relatively few organisms are released into the air compared to breakage that releases the fluid into the centrifuge chamber.

a. Safety Procedures Applicable to All Centrifuging

A safety centrifuge cabinet or safety centrifuge trunnion cup should be used when centrifuging hazardous or infectious substances. When bench-type centrifuges are used in a Biological Safety Cabinet, the glove panel should be in place with the gloves in place or with the ports covered. The centrifuge operation creates air currents that may cause the escape of agent from an open cabinet (17,27,28).

Centrifuge tubes and trunnion cups should be filled and opened in a Biological Safety Cabinet (29). If centrifugation is to be performed outside the cabinet, the safety trunnion cup should be used. After it is filled and sealed, it should be considered potentially contaminated and should be wiped with a cloth soaked in disinfectant or passed through a disinfectant dunk bath. Since some disinfectants are corrosive to centrifuge cups and heads, a rinse of the cup with clean water is desirable after an appropriate contact time has elapsed.

In some situations, in the absence of "O" ring sealed trunnion cup caps, specimens can be enclosed in sealed plastic bags before centrifugation (24). In the event of breakage, however, the plastic bag is likely to be ruptured. Thus, this technique normally only prevents the escape of organisms that contaminated the outside of the cup.

Before centrifuging, eliminate tubes with cracks and chipped rims, inspect the inside of the trunnion cup and correct rough walls caused by erosion or adhering matter, and carefully remove bits of glass and other debris from the rubber cushion (27,29).

A disinfectant should be added between the tube and trunnion cup to disinfect the materials in case of accidental breakage. This practice also provides an excellent cushion against shocks that might otherwise break the tube (27,29). Care must be taken, however, not to contaminate the culture material with the disinfectant. It must be recognized also that the disinfectant may not completely inactivate the infectious material when the tube breaks because of the dilution of the disinfectant and the high concentration and packing of cells.

Avoid pouring the supernatant material from centrifuge tubes. If you must do so, wipe off the outer rim with a disinfectant afterwards; otherwise, in a subsequent step, biohazardous fluid may be spun off as droplets that form an aerosol (27,29). Use of a vacuum system with appropriate in-line safety reservoirs and filters is preferable to pouring from centrifuge tubes or bottles.

If the sediment is packed infectious microorganisms or other hazardous material and must be resuspended in order to minimize the amount of aerosol created, it is better to use a swirling, rotary motion rather than shaking. If vigorous shaking is essential to suspend the material or achieve homogeneity, a few minutes should elapse before opening the container to allow the aerosol to settle. Shaking always contaminates the closure; thus, there is the added hazard of liquids dropping from the closure or running down the outside of the container. A Biological Safety Cabinet with gloves in place may be required to assure safety to the laboratory worker when performing some of these operations.

Avoid filling the centrifuge tube to the point that the rim, cap, or cotton plug becomes wet with culture (27,29).

Screw caps or caps that fit over the rim outside the centrifuge tube are safer than plug-in closures. Some fluid usually collects

between a plug-in closure and the rim of the tube. Even screw-capped bottles are not without risk, however; if the rim is soiled and sealed imperfectly, some fluid will escape down the outside of the tube (20).

Aluminum foil should not be used to cap centrifuge tubes containing toxic or infectious materials because these light-weight caps often become detached or ruptured during handling and centrifuging (20).

The balancing of buckets and trunnion cups is often improperly performed. Care must be taken to ensure that matched sets of trunnions, buckets and plastic inserts do not become mixed. If the components are not inscribed with their weights by the manufacturer, colored stains can be applied for identification to avoid confusion. When the tubes are balanced, the buckets, trunnions and inserts, including any disinfectant solution or water added for balancing, should be included in the procedure. The basic concern is that the centers of gravity of the tubes are equidistant from the axis of rotation. To illustrate the importance of this, two identical tubes containing 20 g of mercury and 20 g of water, respectively, will balance perfectly on the scales; however, their performance in motion is totally different, leading to violent vibration with all its attendant hazards (30).

b. Older Type and Small Portable Centrifuges

Older type centrifuges that do not have aerosol-tight chambers have been shown to allow the escape of aerosol created from various sources:

- biohazardous fluid remaining on the lip of the tube after decanting the supernatant fluid
- leakage from a tube in an angle-head centrifuge resulting from overfilling a tube and placing aslant in the centrifuge
- leakage from nonrigid tubes that distort under centrifugal forces, or
- fluid trapped in the threads of screw caps.

Safety trunnion cups should be used to prevent escape of aerosol in the event the primary culture container held in the cup should break or in any other manner allow the release of agent into the cup. The handling of the culture, the filling of centrifuge tubes and placing them in the safety trunnion cups should be done in a Biological Safety Cabinet. The outside of the trunnion cup should be decontaminated before the cup is removed for centrifuging. Subsequently, the cup should be returned to and opened in a Biological Safety Cabinet. Where applicable, the centrifuge itself should be placed in the cabinet, and, if need be, a cabinet should be specifically constructed for the centrifuge.

Small portable, "clinical" centrifuges have been shown to be hazardous (31). The microhematocrit centrifuge, in particular, has been shown to produce aerosols. A frequent practice is to centrifuge blood samples in tubes without closures or to use cotton plugs secured in the tubes by means of tape or pins. It should be recognized that some tissue specimens contain viable infectious microorganisms, particularly hepatitis virus, and that open tubes, contaminated closures, and release of aerosols from blood samples and tissue suspensions can be hazardous to laboratory personnel.

c. Sharples Centrifuges

Using the Sharples centrifuge with infectious or hazardous materials poses both engineering design and safety problems. The Sharples centrifuge is driven by a steam or air turbine, requires refrigeration around the bowl, and is equipped with feed and effluent lines. It has a continuous feed that could involve large volumes of liquid material, depending on the amount of solids in the material to be handled and the type of bowl. The centrifuge generates a massive aerosol that is almost impossible to contain within the instrument even with a hermetically sealed bowl (23). For these reasons, a ventilated safety cabinet is necessary to enclose the centrifuge. It may be desired to accommodate the material to be centrifuged and the effluent in the cabinet or handle it by means of connectors through the walls of the cabinet. If the rotor must be transferred to another cabinet

after use, it should be passed through a dunk bath, wrapped in a disinfectant-soaked towel, or placed in another container, the outside of which is decontaminated. Decontamination of the centrifuge bowl, lines, and surrounding cabinet can be accomplished by liquid disinfectants, formaldehyde vapor or ethylene oxide, followed by additional cleaning and rinsing. The rotor can be steam sterilized.

d. High-Speed Centrifuges

Centrifugation at high speeds presents additional hazards because of the higher stresses and forces applied to components of the system. In addition to the recommended practices listed above, precautions should be taken to filter the air exhausted from the vacuum lines, to avoid metal fatigue resulting in disintegration of rotors, and to apply proper techniques in cleaning, handling, and using centrifuge components. Some of these precautions are discussed briefly below.

In high-speed centrifuges, the chamber is connected to a vacuum pump. If there is a breakage or accidental dispersion of infected particles, the pump and the oil in it will become contaminated. A HEPA filter should be placed between the centrifuge and the pump (20).

High-speed rotor heads are prone to metal fatigue, and, where there is a chance that they may be used on more than one machine, each rotor should be accompanied by its own log book indicating the number of hours run at top or de-rated speeds. Failure to observe this precaution can result in dangerous and expensive disintegration. Frequent inspection, cleaning, and drying are important to ensure absence of corrosion or other damage that may lead to the development of cracks. If the rotor is treated with a disinfectant, it should be rinsed with clean water and dried as soon as the disinfectant has adequately decontaminated the rotor. Rubber "O" rings and tube closures must be examined for deterioration and be kept lubricated with the material recommended by the makers. Where tubes of different materials are provided (e.g., celluloid, polypropylene, stainless steel), care must be taken that the tube closures designed specifically for the type of tube in use are employed. These caps are often similar in

appearance, but are prone to leakage if applied to tubes of the wrong material. When properly designed tubes and rotors are well maintained and handled, leaking should never occur (30).

Cleaning and disinfection of tubes, rotors and other components require considerable care. It is unfortunate that no single process is suitable for all items, and the various manufacturers' recommendations must be followed meticulously if fatigue, distortion and corrosion are to be avoided. This is not the place to catalogue recommended methods, but one less well appreciated fact is worthy of mention. Celluloid (cellulose nitrate) centrifuge tubes are not only highly flammable and prone to shrinkage with age and distortion on boiling, but also can be highly explosive in an autoclave (30).

e. Large-Scale Zonal Centrifuges

Zonal centrifuges have been developed to process relatively large volumes, 5 to 150 liters, of material. The pumps, valves, seals, feed lines, connectors, and vacuum and cooling systems, associated with these centrifuges, as well as the large volumes processed at high speeds, create the potential for leakage and generation of hazardous aerosols leading to the contamination both of the environment and of the operating personnel. The following areas have been identified as the principal sources of potential leakage: the centrifuge lip and face seals, the coolant system, the turbine exhaust air, various lines and connectors, the feed system, fraction collection, and during decontamination. In addition, the possibility of spills occurring during loading, unloading, sample collection, decontamination, and other procedures must be recognized (32,33).

The several seals in the equipment pose the greatest potential for escape of hazardous material because inherent to the system is the necessity for pressurizing the process fluid to obtain flow through the rotor; in addition, leaks may occur because of the large centrifugal forces exerted at all points in the rotating component. Procedural hazards identified include: (a) the danger of snagging or rupturing one of the numerous lines (influent, effluent, rotor by-pass, etc.), particularly when

hemostats are used as clamps; (b) undetected over-pressurization of lines resulting in a rupture of a line or failure of a connection because flow was obstructed by bubbles caught in the system; (c) manual making and breaking of connections during the centrifuge operation; (d) inadequate precautions in handling gradient fractions containing very high concentrations of the purified material; and (e) incomplete or ineffective decontamination procedures of the rotor, feed lines and other components of the equipment before disassembly and cleanup (34-36).

Zonal centrifuges that employ B rotor systems can be readily adapted under Class I cabinets. Some laboratories have attached a fume hood onto the centrifuge and have installed a HEPA filter in the exhaust duct from the hood. This type of arrangement permits utilization of the hood for most activities associated with the centrifugation process. It is suggested that a sink be installed in the hood or a container be available adjacent to the centrifuge for dunk decontamination of the rotor.

Users of these centrifuges feel that the potential hazards can be decreased by designing more dependable lip seals and face seals, by designing a biologically tight coolant system with provisions for adding fresh glycol and withdrawing contaminated coolant in a closed system, and by making the control console switches more versatile, such as the addition of switches to permit coolant flow without the need of having to turn on the vacuum for the post-run flushing to the coolant system.

Today, many laboratories are using the batch or continuous flow zonal type ultracentrifuge. Since each installation may be somewhat different, it may be necessary to determine initially, using a biological simulant, whether leakage occurs and whether there is aerosolization of fluid into the room. In addition, it is advisable to examine the equipment carefully to judge which parts are most likely to leak and take protective measures, if possible. For example, routine part changes and inspections should be done to minimize faulty components with the K-II continuous sample flow zonal centrifuge (28), namely: (a) change top lip seal after each run, (b) change bottom lip seal after three runs, (c) inspect face seal after

each run, (d) change rotor end cap "O" rings after each run, and (e) check rotor spindle pivots after each run to assure a smooth-running rotor. The catcher-slinger drain container should contain iodophor equal to 2% when the container is full, and the coolant reservoir level is checked regularly to determine if seal leakage is occurring.

The following safety precautions should be observed when using large-scale zonal centrifuges:

- The centrifuge normally should be placed in a Biological Safety Cabinet under negative pressure when using hazardous agents. For high risk agents, the centrifuge and satellite apparatus should be contained in a Class III cabinet system. An alternative to the cabinet is a small negative pressure room with a leak-tight door for maintenance access. The centrifuge in this small room could be operated remotely or through glove ports.

- The exhaust air from the containment room or safety cabinet should be filtered with HEPA filters.

- The exhaust air from the turbine drive should be passed through HEPA filters before being released to the atmosphere. Installation of a trap to remove oil mist from this exhaust air markedly extends the life expectancy of the filters. Vacuum pump exhaust air should be similarly filtered before release.

- Although some manufacturers recommend that the catcher-slinger fluid collections be purged through the turbine exhaust, there is an advantage in draining the collections to the exterior of the assembly. The discharge from the drain orifice, both air and fluid, must be contained; a disinfectant-containing transparent receiver with a filtered vent is recommended. A similar trap and venting arrangement should be used on the rotor bypass lines for removing bubbles from the fluid feed lines.

- Process feed lines, especially those under positive pressure, should be kept as short as possible and have as few connections as possible. Utilization of 3-, 4-, and 5-way valves is suggested to facilitate the consolidation of the many lines in the system. A panel consisting of pump, flow meter, pressure gage, valves and stainless steel fluid lines, to which influent and effluent lines are attached, is suggested. For all external flexible lines, tygon tubing with a minimum of 1/16" wall thickness and polypropylene connectors having positive, strong fasteners are suggested. Additional safety is provided by nylon strap hose clamps on slip fittings. Operating pressure on feed lines should not exceed 15 psig. A pressure gage and flow meter should be mounted in the line between

the pump and the split to the top and bottom rotor feed lines. An added safety factor would be the provision of an audible alarm to the pressure gage. If possible, the feed line system should be pressure tested before introduction of biohazardous fluids. Testing is usually performed at the time the face seals are leak tested by filling the system with sterile buffer solution or water and the air is purged from the rotor.

- A peristaltic pump is recommended for energizing the flow of process fluids to the rotor. Although line pressure can be automatically regulated to a preset limit at the air pressure source, the pump method is recommended because it places only the line between pump and rotor under pressure. Because some pumps use soft rubber tubing, ballooning and rupture are possible; this tubing should be replaced frequently. It is recommended that the peristaltic pump, and as many of the lines as possible, be contained in a primary barrier.

- Gradient fractions should be collected within the confines of a primary barrier. A Class I or Class II cabinet is recommended. For high risk agents, a Class III cabinet should be used. An alternative is to perform the fraction collection within a plastic glove bag or box prefitted with the gradient discharge tubing running into it. After the fractions are collected, the entire bag and contents are removed to a safety cabinet for subsequent manipulation.

- The total system, including feed lines, effluent lines, coolant system, and ancillary equipment that may have contained or been exposed to hazardous agents, should be decontaminated prior to any breaking of lines or disassembly of the centrifuge. Gradient residue should be flushed from the rotor and core with warm water before the disinfectant is introduced. The rotor should be filled and flow reversed several times to assure contact of the disinfectant with all surfaces. Surface decontamination of the rotor exterior and shafts is suggested as it is raised from the rotor chamber. The end caps should be loosened and the rotor and core completely immersed in a disinfectant solution for initial cleaning. Special precautions should be taken against accumulations of debris in the shoulders of the end caps that the internal disinfectant may not have reached. All lines, influent bottles, reservoirs, connectors, etc., must be autoclaved before cleaning for reuse or discarding. For high risk agents, the decontamination with disinfectant should be followed by ethylene oxide exposure overnight at 60% relative humidity and 80°F for the entire cabinet and its contents. Ethylene oxide access to the rotor chamber and turbine air drive lines should be provided. After the centrifuge and external equipment (lines, pump, valves, etc.) have been cleaned, reassembled, leak tested, and are ready for the next lot of material, they must be sterilized. For virus work, 70% ethanol is recommended; flow must be reversed several times and all bubbles removed to assure contact of the alcohol with all surfaces. The ethanol is drained and flushed out with sterile distilled water to minimize the possibility of residual concentrations that would

affect the virus. For bacterial and other organisms, particularly those that produce spores, alcohol may not be a suitable disinfectant. Regardless of what disinfectant is used, however, the system should be flushed out with sterile distilled water. In some instances, it may be necessary to flush the system with a sterile specific neutralizer for the disinfectant prior to the final flushing with sterile distilled water.

- Protective clothing, including a respirator, should be worn in areas where there is possible exposure to hazardous or infectious materials.
- No material should be removed from the special centrifuge room or Biological Safety Cabinet unless it has been decontaminated.
- The operator should be thoroughly familiar with this equipment to identify problem areas and to establish a system of replacing components likely to result in leakage.

6. Blenders, Mixers, Sonicators, and Cell Disruption Equipment

Hazardous aerosols are created by most laboratory operations concerned with blending, mixing, stirring, grinding or disrupting materials such as cells, tissues, blood samples, freeze-dried sera, and environmental samples that may contain infectious, toxic or otherwise hazardous materials. Even use of the mortar and pestle can be a hazardous operation. Ball mills, colloid mills, jet mills, tissue grinders, magnetic mixers, stirrers, sonic cleaning devices, ultrasonic cell disintegrators, and shakers are other devices that can produce hazardous aerosols.

a. Blenders and Mixers

The hazards associated with the liberation of aerosols during the operation of the blender have been recognized for many years. In one investigation (37), the air was sampled during the operation of two types of blenders; one was a one-liter plastic-capped bowl, the other was a 500 ml screw-capped bowl. The results, shown below, are indicative of the hazardous nature of aerosol release from a standard blender and also show how rapidly the aerosol decays within the blender.

Serratia indica recovered from aerosols produced during the operation of a blender

	<u>Serratia indica</u> recovered*	
	Plastic cap	Screw cap
During 2-minute operation	511	18
Cap removed immediately after blender was turned off	>2,100	>2,100
Cap removed 5 minutes after blender was turned off	306	629
Cap removed 90 minutes after blender was turned off	50	40

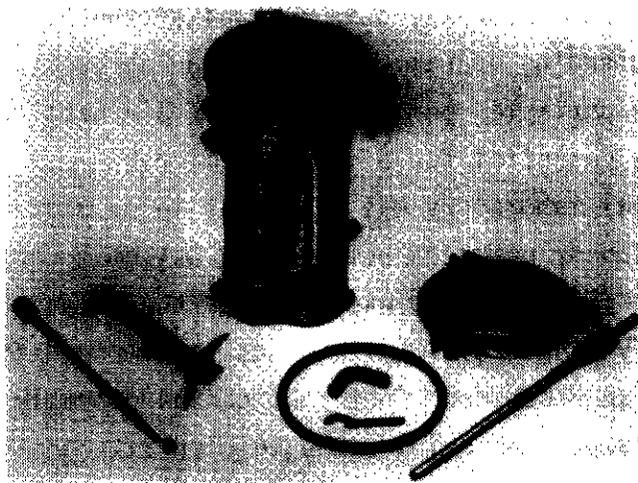
*Numbers refer to colonies appearing on sieve sampler plates. Sampling was at the rate of 1 cu. ft. per min. for 2 min. during blending, and for 3 min. following the operation. When the cap was removed, it was replaced after 10 sec.

Investigations were conducted on the particle size distribution of S. marcescens aerosols created during common laboratory procedures and simulated laboratory accidents (38). Over 1,600 viable particles per cubic foot of air sampled were recovered during blending operations and more than 93% of these particles were less than five microns in size. It was demonstrated that removal of the blender top immediately following the blending operation produced an aerosol with a mean concentration of 1,500 viable particles per cubic foot of air sampled.

The potential for accidental microbial aerosol transmission in the biological laboratory was discussed in 1973 at the Conference of Biohazards in Cancer Research held at the Asilomar Conference Center, Pacific Grove, California; it was reported that blenders and homogenizers are particularly dangerous (39). It was recommended that these devices be used in a hood or other container that can be properly ventilated if the material is suspected of being pathogenic or allergenic. It was stressed that a gastight cabinet must be used in the event the material is highly pathogenic.

High-speed blenders for safely processing infectious materials have been proposed. The unique features of one blender include (i) the elimination of gaskets and bearings at the bottom of the blender bowl by placing the bearings and drive motor at the top, outside the bowl, (ii) the provision for cooling the drive shaft and bearings with dry ice, (iii) the inclusion of a rubber washer in the lid, which is screwed onto the cup, and (iv) the fabrication of the bowl and lid from stainless steel. An air inlet and drainpipe are provided to allow for the removal of the contents without opening the lid, which would release an aerosol. This equipment is not commercially available; however, sufficient detail for fabrication has been published (40). Other studies (41,42) concerned the infection hazards of the high-speed blender and presented means of correcting the problem by utilization of a new blender design. As an outgrowth of these studies, a safety container (Waring AS-1) for the Waring blender is commercially available that can be autoclaved or otherwise sterilized, has biologically inert teflon bearings, a "standpipe"

agitator, a leak proof lid with an "O" ring gasket secured by swivel thumbscrews, and outlet fittings to allow the continuous flow of the ingredients. The lid also has an outlet plug to permit easy removal of samples, as shown in the following figure.



**CONTAINER (WARING AS-1) FOR THE SAFE BLENDING
OF HAZARDOUS MATERIALS**

Magnetic mixers, although generally operated at slower speeds than blenders and not designed to create a turbulent, macerating action, are capable of creating aerosols, particularly if the material mixed produces bubbles or foam. Magnetic mixers provide a comparatively gentle, swirling action, but the mixing of infectious, oncogenic, allergenic, or toxic materials should be considered a potentially hazardous operation. Thus, the same safety concerns apply to magnetic mixers as to blenders.

b. Sonicators

Aerosol hazards associated with the operation of an ultrasonic oscillator have been reported (43,44). Particles of infectious or hazardous materials can escape because of loosely fitting covers, loose gaskets at the bottom of the cup, or when the contents are removed from the cup. Use of a larger size "O" ring was found to provide a satisfactory closure. Modification of the cover to permit installation of a rubber diaphragm provided means for the safer removal of the contents by a syringe and needle. It also was recommended that sonicators be used in Biological Safety Cabinets.

Adequate decontamination of equipment potentially contaminated with infectious material prior to sonic cleaning is essential because of the hazard of creating aerosols during the sonic treatment (45). Wherever sonicators are used in a cleaning process, such as in dishwashers, animal cage washers, etc., all items should be sterilized prior to cleaning.

c. Recommended Practices

Safe laboratory practices that are required generally when using blenders, mixers, ultrasonic disintegrators, colloid mills, jet mills, grinders, and mortars and pestles with hazardous biological or chemical materials are as follows:

- Operate blending and cell disruption and grinding equipment in a Biological Safety Cabinet.
- Use safety blenders designed to prevent leakage from the rotor bearing at the bottom of the bowl. In the absence of a leakproof rotor, inspect the rotor bearing at the bottom of the blender bowl for

leakage prior to operation. Test it in a preliminary run with sterile water, saline or methylene blue solution prior to use.

- If the blender is used with infectious material, use a towel moistened with disinfectant over the top of the blender. Sterilize the device and residual contents promptly after use.

- Glass blender bowls are undesirable for use with infectious material because of potential breakage. If used, they should be covered with a propylene jar to prevent dispersal of glass in the event the bowl breaks.

- A heat-sealed flexible disposable plastic film enclosure can be used for a grinder or blender. The safest practice is to use these within a Biological Safety Cabinet. That means they are not used for total containment but rather to spare gross contamination of the cabinet when equipment or procedures are used that are known to release aerosols.

- Blender bowls sometimes require supplemental cooling to prevent destruction of the bearings and to minimize thermal effects on the product.

- Before opening the safety blender bowl, permit the blender to rest for at least one minute to allow settling of the aerosol cloud.

7. Miscellaneous Precautions and Recommendations

Water baths and Warburg baths used to inactivate, incubate, or test infectious substances should contain a disinfectant. For cold water baths, 70% propylene glycol is recommended (29,46). Sodium azide should not be used as a bacteriostatic. It creates a serious explosion hazard.

Deepfreeze, liquid nitrogen, and dry ice chests and refrigerators should be checked and cleaned out periodically to remove any broken ampoules, tubes, etc., containing infectious material, and decontaminated. Use rubber gloves and respiratory protection during this cleaning. All infectious or toxic material stored in refrigerators or deepfreezers should be properly labelled. Security measures should be commensurate with the hazards (29,46,47). The degree of hazard represented by contaminated liquid nitrogen reservoirs will be largely dependent upon the infectious potential of the stored microorganisms, their stability in liquid nitrogen, and their ability to survive in the airborne state. Investigations suggest that storing tissue culture cell lines in containers other than sealed glass ampoules might result in potential intercontamination among cell lines stored in a common liquid nitrogen repository.

It must be recognized that evacuating the atmosphere from a vacuum steam sterilizer prior to sterilization of contaminated material potentially can create a hazard by releasing infectious material to the atmosphere. This hazard can be prevented by installation of an efficient in-line HEPA filter (water resistant; e.g., Flanders 7C81R-G) (48).

Ensure that all hazardous fluid cultures or viable powdered infectious materials in glass vessels are transported, incubated, and stored in easily handled, nonbreakable leakproof containers that are large enough to contain all the fluid or powder in case of leakage or breakage of the glass vessel (29,46).

All inoculated petri plates or other inoculated solid media should be transported and incubated in leakproof pans or leakproof containers (29,46).

Care must be exercised in the use of membrane filters to obtain

sterile filtrates of infectious materials. Because of the fragility of the membrane and other factors, such filtrates cannot be handled as non-infectious until culture or other tests have proved their sterility (29,46).

Shaking machines should be examined carefully for potential breakage of flasks or other containers being shaken. Screw-capped durable plastic or heavy walled glass flasks should be used. These should be securely fastened to the shaker platform. An additional precaution would be to enclose the flask in a plastic bag with or without an absorbent material.

No person should work alone on an extremely hazardous operation (29,46).

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B. Personal Hygiene Habits and Practices

Personal hygienic practices in the laboratory are directed, in most part, toward the prevention of occupationally acquired physical injury or disease. To a less obvious extent, they can raise the quality of the laboratory work by reducing the possibilities for contamination of experimental materials. The reasons for many of the recommended precautions and practices are obvious, but, in some instances, amplification will permit a better review of the applicability to any one specific laboratory. Consequently, what might be forbidden in one laboratory might be only discouraged in another and be permissible in a third. Nevertheless, adherence to safe practices that become habitual, even when seemingly not essential, provides a margin of safety in situations where the hazard is unrecognized. The history of occupational injury is replete with examples of hazards unrecognized until too late. The following guidelines, recommendations, and comments are presented with this in mind:

- Food, candy, gum, and beverages for human consumption should be stored and consumed only outside the laboratory.
- Foot-operated drinking fountains should be the sole source of water for drinking by human occupants of the laboratory (1).^{*} These should be located in the corridor, not the laboratory. The water line that serves the laboratory faucets should be separated from the water line to the drinking fountain by appropriate devices that prevent back-flow.
- Smoking is not permitted in the laboratory or animal quarters. Cigarettes, pipes, and tobacco should be kept only in clean areas designated for smoking (2,3,4).
- Shaving and brushing of teeth should not be permitted in the laboratory. Razors, toothbrushes, toiletry supplies, and cosmetics are permissible in clean change rooms or other clean areas, but should never be used until after showering or thorough washing of the face and hands.
- A beard may be undesirable in the laboratory in the presence of actual or potential airborne contamination because it retains particulate contamination more persistently than clean-shaven skin. A clean-shaven face is essential to the adequate fit of a face mask or respirator when the work requires respiratory protection (1,3).

^{*}The references for this section appear on page 45.

- Keeping hands away from mouth, nose, eyes, face, and hair should become habit. This may prevent self-inoculation (1,3).

- For product protection, persons with long hair should wear a clean hair net or head cover. This has long been a requirement in hospital operating rooms and in the manufacture of biological pharmaceutical products. A head cover also will protect the hair from fluid splashes, from swinging into Bunsen flames and petri dishes, and will reduce facial contamination caused by frequent rearrangement of the hair to move it off the face (3).

- Long-flowing hair and loose-flapping clothing are dangerous in the presence of open flame or moving machinery. Rings, wrist watches, and other jewelry also are physical hazards during the operation of some types of machines (2,3).

- Personal items, such as coats, hats, storm rubbers or overshoes, umbrellas, purses, etc., should not be kept in the laboratory (5).

- Books and journals returnable to the institutional library should be used only in the clean areas as much as possible. Under no circumstances should books or journals on loan from institutional libraries be taken into a P4 facility (29,42).

- Personal cloth handkerchiefs should not be used in the laboratory. Cleansing tissue should be available in laboratories and change rooms.

- Hand washing should be done promptly after removing protective gloves. Tests show it is not unusual for microbial or chemical contamination to be present despite use of gloves, due to unrecognized small holes, abrasions, tears, entry at the wrist, or solvent penetration through the gloves.

- Hands should be washed after removing soiled protective clothing, before leaving the laboratory area, before eating, before smoking, and throughout the day at intervals dictated by the nature of the work. Jewelry should not be worn in the laboratory as it will interfere with the hand washing procedure. If worn, it could become contaminated and cause the contamination to be brought to the home (6,39,43,45).

- A disinfectant wash or dip may be desirable in some cases, but its use must not be carried to the point of causing roughening, desiccation or sensitization of the skin (6,39,43).

- Work should not be done with biohazardous materials by anyone with a fresh or healing cut, abrasion, a lesion of the skin or any open wound, including that resulting from a tooth extraction (6,45).

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C. Protective Clothing and Equipment

Protective clothing and equipment are used to protect the laboratory worker from contact with infectious, toxic and corrosive agents, excessive heat, fire, and other physical hazards. Also, suitable clothing and equipment can help protect the experiment itself from contamination. The extent and kinds of clothing and equipment to be selected for any particular activity are dependent upon the research operations and the levels of potential hazard associated with them. While clothing and personnel safety equipment are of importance in an overall biological safety program, they are to be used with the understanding that they serve as a secondary line of defense. Biological Safety Cabinets accompanied by good laboratory techniques and procedures are the primary barriers against potential exposure to hazardous materials.

Certain types of laboratory clothing and protective equipment are safer, more practical, and provide greater comfort than others. Comfort, however, must not be the overriding factor in the final selection of an item required for protection. Once proper protective clothing and equipment are selected, it is the task of the supervisor to provide training in their use and to assure that employees properly use and maintain them.

The applications of these various items of protective clothing and equipment in the conduct of recombinant DNA research are summarized in the table on the following page.

**SUMMARY OF MINIMUM REQUIREMENTS REGARDING LABORATORY ISSUED
PROTECTIVE CLOTHING AND EQUIPMENT FOR RECOMBINANT DNA RESEARCH**

Physical Containment Level	Protection Required				
	Hand	Head	Body	Foot	Respiratory
P1			The use of laboratory gowns, coats or uniforms is at the discretion of the laboratory supervisor.		
P2			The use of laboratory gowns, coats or uniforms is required. Laboratory clothing shall not be worn to the lunchroom or outside the building in which the laboratory is located.		
P3	Gloves shall be worn when handling materials requiring P3 containment. They shall be removed aseptically immediately after use and decontaminated.		Laboratory clothing that protects street clothing (i.e., long-sleeve, solid-front or wrap-around gowns, no-button or slip-over jackets, etc.) shall be worn in the laboratory. Front button laboratory coats are unsuitable. Laboratory clothing shall not be worn outside the laboratory and shall be decontaminated before it is sent to the laundry.		Respiratory protection is required for emergency procedures.
P4	Gloves shall be worn when conducting procedures requiring P4 containment.	Headcover is required. Headcover is not to be worn outside the work area.	Complete laboratory clothing, including undergarments, pants and shirts, or jumpsuits, shall be provided and worn in the P4 facility. Laboratory clothing shall not be worn outside the P4 facility.	Laboratory-issued shoes are required. These are not to be worn outside the work area.	Respiratory protection is required for emergency procedures.

For P4 suit room conditions, all personnel are required to wear one-piece positive pressure suits.

The specifics of protective clothing and equipment are included in the following discussions; however, a broader treatment of the overall field can be found in the Accident Prevention Manual for Industrial Operations, Chapter 19, "Personal Protective Equipment," prepared by the National Safety Council, pages 465-527, 7th edition, dated 1974.

1. Laboratory Clothing

Laboratory clothing can serve to protect the wearer, the experiment, and the environment against contamination. The user must wear the garments in the manner intended to assure the benefits of the protection they can provide. If proper precautions are not taken, clothing may carry microbiological contamination outside the laboratory and into other work areas, cafeterias, or the home. Microorganisms can remain viable on cotton and wool fabrics and can be disseminated from these fabrics.

The National Institutes of Health Recombinant DNA Guidelines include requirements for the use of protective clothing and equipment at the P2, P3 and P4 physical containment levels. For P1 containment, the use of laboratory clothing is left to the discretion of the project supervisor. However, if good microbiological practices are to be applied to protecting the integrity of the experiment, the general and effective use of laboratory clothing should be encouraged.

Local clothing requirements above the minimum required by the Guidelines will vary from one institution to another. Many institutions recommend that animal handlers and technical operations personnel be provided a complete clean clothing change on a daily basis. While a full-length and fully fastened laboratory coat worn over street clothing may be acceptable in some cases, laboratories have found that, for reasons of comfort, mobility and enhanced protection, one- or two-piece laboratory suits, solid-front gowns, and wraparound smocks are preferable. Long-sleeved garments are best for protection of the arms and to minimize shedding of contaminating microorganisms from them. For the same reasons, consideration should be given to the need for a head covering (cap or head hood) and a snug-fitting collar at the neck. The garments usually have close-fitting closures: knitted cuffs, snaps, drawstrings or elastic circlets. Drawstrings for men's pantwaist closures and adjustable snap-type waist closures for women seem to be preferred. Some of the available styles of laboratory clothing are shown in the illustrations later in the Section.

Clothing inventory planning should provide for the needs of visitors

and of maintenance and security personnel. For some P1, P2 and P3 facilities, scientists who visit on a short-term basis may find long-sleeved wraparound disposable smocks to be versatile in that they give good protection over street clothes and accommodate a range of sizes. Other laboratories use disposable jumpsuits. These types also are used for the short visits that maintenance staff and security guards make for routine visits in off-duty hours to check that equipment is performing satisfactorily. When extensive maintenance work is involved, the personnel may require sturdier, reusable garments.

Laboratory-issued clothing should not be worn outside the facility or to the library, cafeteria, or other places accessible to the public. For P3 and P4 facilities, the use of specially colored laboratory clothing is recommended as part of control practices on the movements of personnel, the sterilization and laundering practices, and the disposal of these garments.

Both reusable and disposable laboratory clothing are available from supply houses. Reusable clothing, although initially more expensive, has the potential of longer life. It should be of a quality capable of withstanding various and repeated decontamination and laundry treatments. Disposable clothing does not have this attribute; however, it has its place in those situations where visitors to the laboratory must be issued clothing, and also in those situations where decontamination facilities, such as autoclaves or ethylene oxide sterilizers, are too distant or not readily available. When there is the potential for contamination of the laboratory clothing with hazardous chemicals, the use of disposable articles allows for degradation by incineration.

Reusable laboratory clothing is made principally of cotton and polyester and combinations thereof. Nylon also is used, but is not recommended for clothing that must be autoclaved. Some of the factors that must be considered in the selection of appropriate laboratory garments are comfort, impenetrability, stitching, appearance, type and effectiveness of closures, shrinkage (not to exceed 1%), antistatic properties, style, color, and ability to withstand repeated autoclaving at 250°F. A fabric composed of 65% polyester, 34% cotton and 1% stainless steel metal fibers

(antistatic) has been widely used. Consideration also must be given to the weight of the material used. Heavier fabrics may be required for rough service or work done in cooler atmospheres, as in the case of animal handlers.



FULLY BUTTONED
LABORATORY COAT



WRAPAROUND SMOCK



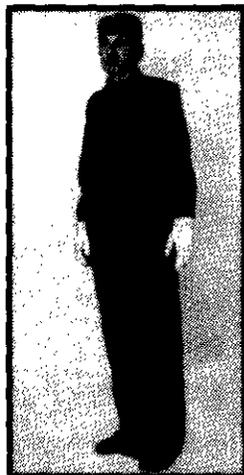
SOLID FRONT GOWN



ONE-PIECE
LABORATORY SUIT



TWO-PIECE
LABORATORY SUIT



HEAVY DUTY COVERALL

TYPES OF LABORATORY CLOTHING

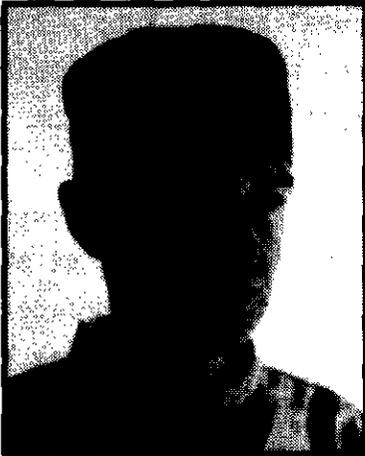
Disposable laboratory clothing, normally, can be obtained from the same companies that supply the reusable garments. Although manufacturers claim that rapidly rising labor costs for decontaminating and laundering make purchase of disposable clothing attractive in contrast to the reusable type, it is up to the individual organization utilizing the clothing to determine which is economical for the intended use. In actual practice, some laboratories report that durable-quality reusable clothing is less expensive.

Two of the popular materials used in the manufacture of disposable garments are made, in one case, from a synthetic polyethylene fiber and, in the second, from cellulose fibers. The synthetic fiber is woven and the cross-fibers are then bonded by heat under pressure. The material so formed has high strength (wet or dry) and presents a good barrier (wet or dry) to movement of particles through it while retaining reasonable breathability for comfort. Its strength permits several days' use of garments under conditions of reasonable activity and wear. The cellulose materials are formed by layering the fibers and bonding the layer to a scrim of nylon mesh. Normally absorbent, the material can be obtained in treated, fluid repellent form. The porosity of this material assures that it breathes. For any of these fabrics, inquiry should be made of the supplier or manufacturer as to their resistance to solvents and solutions of various salts at different pH's in relation to their intended use. Polyethylene generally provides resistance to a broad spectrum of solvents, cellulose fibers less so dependent, in part, upon the surface treatment they are given. Consideration should be given to special needs for self-extinguishing fabrics.

All reusable clothing worn in P1 and P2 facilities can be discarded into a closed container and laundered in the conventional manner if it is not overtly contaminated. Reusable clothing that is from a P3 or P4 facility should be placed in a closed container and subsequently sterilized before laundering. Any clothing, including that from a P1 facility, that may have been overtly contaminated should be wetted down with a disinfectant and autoclaved immediately.

All disposable clothing worn in a P1 facility should be discarded

into a closed container with the other noncontaminated laboratory waste materials and discarded. Disposable clothing that has been used in a P2, P3, or P4 facility should be placed in a closed container and subsequently autoclaved prior to discarding. Again, if it has been overtly contaminated, it should be wetted down with a disinfectant and autoclaved immediately.



SIMPLE CAP



BOUFFANT CAP



HOODED CAP

TYPES OF HEADCOVERING

2. Gloves, Shoes and Aprons

Gloves, shoes and aprons are important items of safety equipment. Gloves must be comfortable and of sufficient length to prevent exposure of the wrists and forearms. Depending on the intended use, the composition and design of a glove may be required to provide dexterity, strength, low permeability, resistance to penetration by sharp objects, and protection against heat and cold. Quality assurance is an important aspect in glove manufacture, and numerous laboratories have experienced difficulty in obtaining leak-proof rubber gloves. Protective footwear is required where there exists the possibility of injury to the feet. Shoes with protective guards or steel toes and capable of resisting penetration of corrosive or hot liquids are available from several safety supply houses. A change to work shoes is beneficial in laboratories handling microbiological materials. This serves to reduce the amount and type of contamination introduced by street shoes and minimizes the possibility of bringing to the home microbiological contamination from the laboratory. Aprons are worn in conjunction with a laboratory coat or suit to minimize penetration of liquid spill or solid particles through the garment to the body surface. They are particularly useful in laboratories handling chemicals. They also are needed in animal handling facilities where washdowns are routinely carried out and in laboratory dishwashing operations where materials are handled in the presence of steam and hot water. Best protection is provided by a solvent resistant, long apron.

a. Gloves

No one glove can be expected to be satisfactory for all applications. Gloves may be fabricated of cloth, leather, natural (latex) and synthetic (neoprene) rubbers, and plastics. New formulations of synthetic rubbers and plastics continue to be developed as research makes varied and changing demands on the protective capabilities of gloves. Changing applications lead to improved capabilities of low permeability, greater strength, flexibility, tactile sense and control. Even within the

modest laboratory, the requirements to wear gloves may be such that no less than four or five kinds of protective gloves need to be stocked and used.

The type of glove selected is dependent upon the specific activity. For example, delicate work requires the use of thin-walled gloves. Heat-resistant gloves and mittens are an absolute necessity in biomedical laboratories for such operations as handling hot glassware or dry ice. Leather-gauntlet gloves are frequently used when handling certain animals, such as monkeys. For some glove styles, the leather is metal-reinforced to increase protection against animal bites. Other gauntlet-type gloves are needed to protect hands and arms in washroom operations and in working with hazardous chemicals.

Gloves should be worn when working with materials requiring P3 and P4 containment and with toxic substances, and as protection against harmful solvents, acids and caustics. If a glove is to provide protection, it must be of a composition that limits penetration and possesses sufficient strength to maintain the integrity of the barrier under stresses to which the glove is subjected. The data in the table provide some indication of the overall performance characteristics of different glove materials. To achieve tactile sense and control, and sometimes for economic reasons, strength may be compromised by reducing the wall thickness (weight) of gloves. Disposable gloves of rubber and plastic may have wall thicknesses on the order of 1.25 to 6.0 mils. Surgical-type gloves principally range from 8 to 10 mils. For work in Class III cabinets, arm-length gloves of neoprene in thicknesses of 15 to 30 mils have been found to be satisfactory. Heavy-duty industrial gloves are 16 to 40 mils thick. Tearing is frequently experienced with the lighter-weight gloves. Some disposable gloves present problems of fit. Surgical gloves of about 9 mils thickness, sized and shaped for the hand, are normally used when tactile sensitivity is required. Canvas, leather, or heat-resistant gloves should be worn over rubber gloves when handling animal cages or other sharp-edged or hot equipment to prevent tears in the gloves and to protect the skin.

The wearing and final disposal of gloves call for thought and care. Operations in open-front safety cabinets should be preplanned

CHEMICAL RESISTANCE CHART

CHEMICAL	RUBBER	NEOPRENE	BUNA N	POLY VINYL CHLORIDE	POLY VINYL ALCOHOL	CHEMICAL	RUBBER	NEOPRENE	BUNA N	POLY VINYL CHLORIDE	POLY VINYL ALCOHOL
ACIDS MINERAL						SOLVENTS, CHLORINATED					
Chromic.....	P	F	F	G	NR	Carbon Tetrachloride.....	P	F	G	F	S
Hydrochloric 30%.....	G	E	E	G	NR	Chlorobenzene.....	P	F	G	F	S
Hydrofluoric 10%.....	G	E	E	G	NR	Chloroform.....	P	F	G	F	S
Muriatic.....	G	E	E	G	NR	Chloroform: TCE.....	P	F	G	F	S
Nitric 70%.....	F	E	E	F	NR	Dichlorobenzene.....	NR	F	F	F	S
Nitric 10%.....	F	E	E	F	NR	Dichloroethane.....	NR	F	F	F	S
White Fuming Nitric.....	P	P	P	P	NR	Methyl Chloride.....	P	F	G	F	S
Sulfuric 95%.....	G	E	E	G	NR	Perchloroethylene.....	P	F	G	F	S
Sulfuric 10%.....	G	E	E	G	NR	Perchloroethylene.....	P	F	G	F	S
Sulfuric 48%.....	G	E	E	G	NR	Trichloroethylene TCE.....	P	F	G	F	S
Perchloric.....	G	E	E	G	NR	SOLVENTS, PETROLEUM					
Phosphoric.....	G	E	E	G	NR	Bulkane.....	F	E	E	F	S
Perming.....	G	E	E	G	NR	Gasoline.....	F	E	E	F	S
ACIDS ORGANIC						Hexane.....	F	E	E	F	S
Oitic.....	E	E	E	E	NR	Isobutane.....	F	E	E	F	S
Formic.....	E	E	E	E	NR	Kerosene.....	F	E	E	F	S
Lactic.....	E	E	E	E	NR	Naphtha.....	F	E	E	F	S
Linolic.....	E	E	E	E	NR	Petroleum Spirits.....	F	E	E	F	S
Maleic.....	E	E	E	E	NR	Mineral Spirits.....	F	E	E	F	S
Oxalic.....	E	E	E	E	NR	SOLVENTS, MISCELLANEOUS					
Palmitic.....	E	E	E	E	NR	Acetone.....	G	F	F	F	G
Phenol.....	E	E	E	E	NR	Amyl Acetate.....	G	F	F	F	G
Stearic.....	E	E	E	E	NR	Benzene Oil.....	G	F	F	F	G
Tannic.....	E	E	E	E	NR	Carbon Disulfide.....	G	F	F	F	G
ALCOHOLS						Cellar Solvent Acetate.....	G	F	F	F	G
Butanol.....	E	E	E	E	NR	Cyclohexanol.....	G	F	F	F	G
Ethyl.....	E	E	E	E	NR	Dimethyl Formamide.....	G	F	F	F	G
Ethanol.....	E	E	E	E	NR	Ethyl Acetate.....	G	F	F	F	G
Isobutyl.....	E	E	E	E	NR	Ethyl Ether.....	G	F	F	F	G
Isopropyl.....	E	E	E	E	NR	Ethyl Formate.....	G	F	F	F	G
Isopropyl.....	E	E	E	E	NR	Formaldehyde.....	G	F	F	F	G
Isopropyl.....	E	E	E	E	NR	Furfural.....	G	F	F	F	G
Methanol.....	E	E	E	E	NR	Methyl Bromide.....	G	F	F	F	G
Methyl.....	E	E	E	E	NR	Methylene Chloride.....	G	F	F	F	G
Wood.....	E	E	E	E	NR	Methyl Cellosolve.....	G	F	F	F	G
Oxyl.....	E	E	E	E	NR	Nitro Propane.....	G	F	F	F	G
ALDEHYDES						Nitro Benzene.....	G	F	F	F	G
Acetaldehyde.....	G	E	E	G	F	Nitro Toluene.....	G	F	F	F	G
Benzaldehyde.....	F	F	F	F	NR	Propyl Acetate.....	G	F	F	F	G
Formaldehyde.....	F	F	F	F	NR	Tetrahydrofuran.....	G	F	F	F	G
CAUSTICS						Triethylamine.....	G	F	F	F	G
Ammonium Hydroxide.....	E	E	E	E	NR	MISCELLANEOUS CHEMICALS					
Potassium Hydroxide 50%.....	E	E	E	E	NR	Animal Hardener.....	F	E	E	E	E
Sodium Hydroxide 50%.....	E	E	E	E	NR	Carbon Disulfide.....	F	E	E	E	E
AMINES						Castor Oil.....	F	E	E	E	E
Aniline.....	F	F	F	F	NR	Coal Tar Sealer.....	F	E	E	E	E
Diethylamine.....	G	E	E	G	NR	Cotton Seed Oil.....	F	E	E	E	E
Hydrazine.....	G	E	E	G	NR	Cresol.....	F	E	E	E	E
Methylamine.....	G	E	E	G	NR	Cutting Oil.....	F	E	E	E	E
Morpholine.....	E	E	E	E	NR	Diethylamine.....	F	E	E	E	E
SOLVENTS, AROMATIC						Dibutyl Phthalate.....	F	E	E	E	E
Triethanolamine.....	G	E	E	G	NR	Epoxy Resins, Dry.....	F	E	E	E	E
Benzol.....	NR	P	P	P	NR	Hydraulic Fluid.....	F	E	E	E	E
Coal Tar Distillate.....	NR	P	P	P	NR	Petroleum Base.....	F	E	E	E	E
Standard Solvent.....	P	P	P	P	NR	Hydrogen Peroxide 30%.....	F	E	E	E	E
Toluene.....	NR	P	P	P	NR	Linseed Oil.....	F	E	E	E	E
Toluol.....	NR	P	P	P	NR	Mineral Oil.....	F	E	E	E	E
Xylol.....	NR	P	P	P	NR	Mineral Oil.....	F	E	E	E	E
SOLVENTS, KETONIC						Mineral Oil.....	F	E	E	E	E
Acetone.....	E	G	G	E	NR	Mineral Oil.....	F	E	E	E	E
Diisobutyl Ketone.....	F	P	P	F	NR	Mineral Oil.....	F	E	E	E	E
Methyl Ethyl Ketone.....	F	P	P	F	NR	Mineral Oil.....	F	E	E	E	E
Methyl Isobutyl Ketone.....	E	G	G	E	NR	Mineral Oil.....	F	E	E	E	E

KEY TO CHEMICAL CHART:

S—Fluid has no effect E—fluid has very little effect F—fluid has minor to moderate effect G—fluid has moderate to severe effect NR—no noticeable effect (NR—Not suitable for use in fluid)

NOTE: To jobify, the best grade for each table condition is indicated. When selecting physical conditions are involved. Then select the glove with the highest ratings for those conditions

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NOTE: To jobify, the best grade for each table condition is indicated. When selecting physical conditions are involved. Then select the glove with the highest ratings for those conditions

COATED GLOVES PHYSICAL PERFORMANCE CHART

PHYSICAL PERFORMANCE	HYDRON	NEOX	REDMONT	SCORPIO	GRABIT	SNORKEL	GRAPPLER	GRIP	MORKEY
Abrasion Resistance	E	F	G	F	F	F	S	S	F
Cut Resistance	E	E	S	G	E	F	F	F	F
Dry Grip	E	G	G	E	E	E	G	E	G
Flexibility	E	G	G	F	S	F	E	F	F
Puncture Resistance	E	E	E	E	E	E	G	G	F
Wet Grip	F	F	F	S	G	E	E	F	F

KEY TO CHART: S—Superior E—Excellent G—Good F—Fair P—Poor

Courtesy of: Edmont Wilson

UNSUPPORTED GLOVES PHYSICAL PERFORMANCE CHART

PHYSICAL PERFORMANCE	HYDRON	NEOX	REDMONT	SCORPIO	GRABIT	SNORKEL	GRAPPLER	GRIP	MORKEY
Abrasion Resistance	E	F	G	F	F	F	S	S	F
Cut Resistance	E	E	S	G	E	F	F	F	F
Dry Grip	E	G	G	E	E	E	G	E	G
Flexibility	E	G	G	F	S	F	E	F	F
Puncture Resistance	E	E	E	E	E	E	G	G	F
Wet Grip	F	F	F	S	G	E	E	F	F

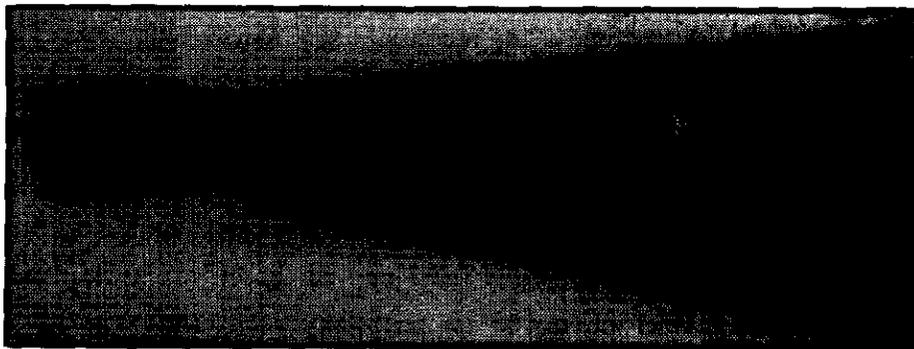
KEY TO CHART: S—Superior E—Excellent G—Good F—Fair P—Poor

so that, once gowned, gloved, seated and with hands and arms in the cabinet, the operator does not have to withdraw from the cabinet until the work has been completed.

Gloves should overwrap the cuff and lower sleeve of the laboratory garment. For further protection of the sleeve, a long glove or a simple disposable plastic arm shield can be used. Armshields can be cut from a roll of polyvinyl chloride or polyethylene; they can be secured at the wrist by the glove. If gloves become overtly contaminated when working in a cabinet, they should be removed and discarded in a waste container in the cabinet with disinfectant sufficient to cover the gloves. New gloves should be available so that work can continue. If the work period is long, it is wise to wipe the gloved hands with disinfectant from time to time.

Arm-length gloves secured to fixed port openings are a requirement in Class III safety cabinets and dry boxes. For this, gloves made of neoprene in thicknesses of 15 to 30 mils have been found to be satisfactory. However, they must be inspected for pinhole leaks as received from the manufacturer and at routine intervals of use. Some manufacturers will give assurance that their gloves have been tested by resistance to high voltage for evidence that they are free of thin areas and pinholes. After intervals of service, and after sterilization of the cabinet system, the gloves, while still attached to the cabinets, should be examined for leaks using the soap bubble test following the certification procedures for Class III Cabinet Systems (III, B, 5).

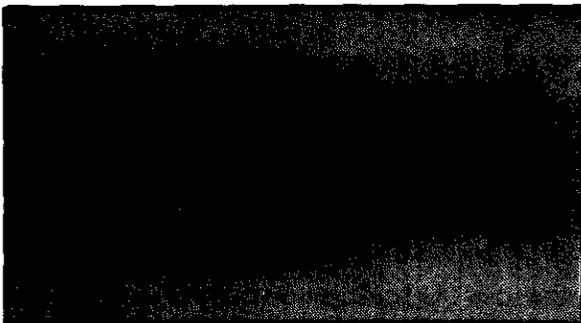
Decontamination practices for gloves depend upon circumstances. Many activities require that gloves be sterile before use. Surgical procedures on experimental animals require the use of sterile gloves. Many laboratories involved in this kind of program will routinely sterilize all gloves before use. Ethylene oxide or formaldehyde gas sterilization can be used for this purpose. Following treatment, the gloves should be thoroughly aerated in flowing filtered air at 21°C or higher for a minimum of 24 hours to prevent skin burns and irritation from residual disinfectants.



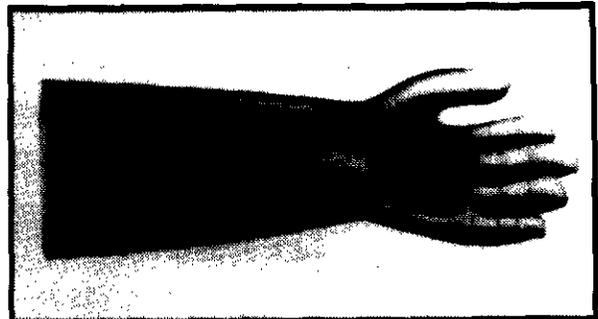
SAFETY CABINET



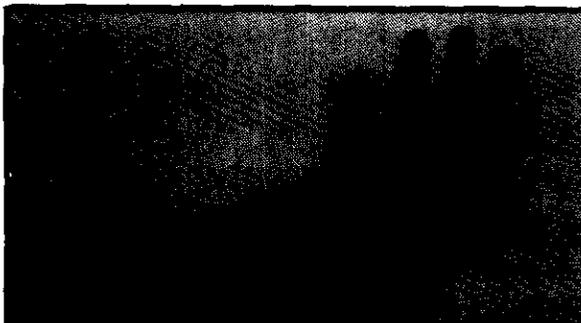
ANIMAL HANDLER (WITH METAL REINFORCEMENTS)



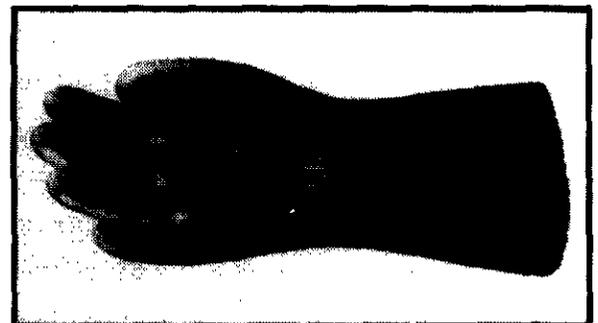
SURGEON'S*



GAUNTLET*



DISPOSABLE*



GRIP*

GLOVES

*Courtesy of Edmont Wilson

The same procedure can be used for gloves of heavy canvas, leather, or coated material, and for heat-resistant gloves. Heavy-weight, reusable rubber and plastic gloves should be placed in a disinfectant solution following use. After thorough contact of proper duration, they can be washed, rinsed, dried, and stored for reuse. The light-weight disposable gloves and the medium-weight (9 mil) surgical gloves, after use, should be discarded into a covered contaminated waste container for eventual autoclaving.

b. Shoes

Laboratory-issued, steel-toed safety shoes or protective boots should be worn by animal handlers and any workers handling heavy items or corrosive chemicals, whether this occurs in an infectious disease research area or in any other laboratory area. Cage racks, cages, dishwashing trays, and gas cylinders are examples of heavy items commonly handled in laboratory activities that require foot protection. Painful accidents and lost-time absences can result from foot injuries. The complete clothing change required for P4 facilities includes a change to laboratory-issued shoes. "Clean room" activities may require special-issue shoes to protect the experiment and sensitive equipment.

All safety or special-issue shoes and boots used in controlled access areas should be identified so that they can be segregated from other special-issue safety shoes used for work in other areas. Special markings, such as painted toes, can be used to identify shoes worn in biohazard areas. If this is done in a light background color, then numbers or initials can be painted on the background color to identify the wearers. It will be necessary to keep on hand extra shoes in some range of sizes for use by the maintenance staff and visitors.

In the event of overt contamination, the shoes should be sterilized promptly by ethylene oxide or formaldehyde gas. Precautionary decontamination by ethylene oxide or formaldehyde gas should be done on a regularly scheduled routine. Exposing shoes on an ultraviolet (UV) rack or wiping them with a suitable liquid disinfectant such as 8% formalin,

2% iodophor, or 2% phenolic compounds may also be used for decontamination of footwear. However, where there is a known or suspect release of biological material, ethylene oxide or formaldehyde gas treatment is recommended as the most dependable means of decontamination. Whenever a liquid or gaseous disinfectant is used, it is necessary to remove all traces of it following application, to avoid allergic skin reactions to possible burns.

c. Aprons

The penetration of hazardous liquids or particulates to, or through, laboratory clothing can be minimized by the utilization of a solvent resistant, long apron. Plastic or rubber aprons worn over the laboratory garment will provide additional protection. Aprons also are required where equipment is handled in the presence of steam and hot water. Full-length aprons and trousers with cuffs worn outside of shoes and boot tops are recommended.

3. Face and Eye Protection

Protection of the face and eyes is of prime importance in laboratories. The possibility is frequently present of impact of foreign material, both liquid and solid, on the head, face, and eyes or on contact lenses. A vast array of face shields, head hoods, protective goggles, and lenses is available from safety supply houses. The selection is dependent upon materials of construction, fit, comfort, compatibility with the disinfectant used, and overall facial area of protection required.

a. Face Protection

Face protection against impact and splash can be obtained through the use of face shields and hoods. They serve to protect the face and neck from flying particles and sprays of hazardous materials; however, they do not provide basic eye protection against impacting objects. Shields should be of such design that they cover the entire face, permit tipping back to clear the face, if desired, and are easily removable in the event of an accident. Hoods are hot to wear unless they are obtained with air lines to supply a cooling flow of air.

b. Eye Protection

Protection to the eyes is an extremely important matter. Microbiologists and virologists may use chemicals that can cause blindness if splattered into the eye. For example, concentrated quaternary disinfectants splashed in the eye in the course of preparing use-dilutions can cause blindness. Personnel must be cautioned as to this danger and given instruction in use of personal protection for eyes, face and hands. In addition, infection can occur through the conjunctiva of the eyes if a pathogenic microorganism is splattered into the eye.

The supervisor has the responsibility of determining that an eye hazard exists, placarding the area as an "Eye-Hazard Area," determining the type of protection required, and ensuring that the appropriate eye protection equipment is available and worn by the employees. For further

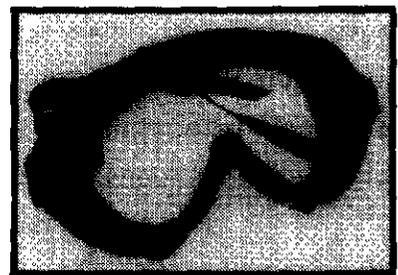
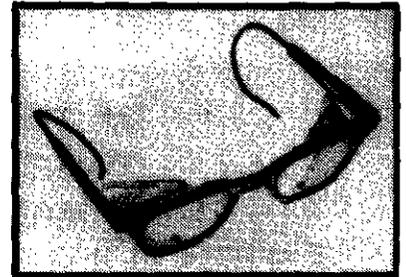
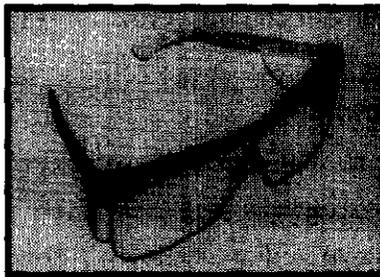
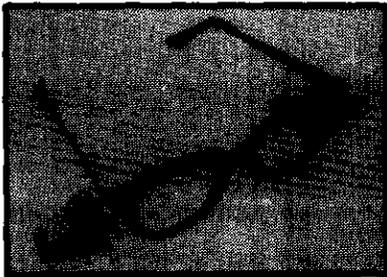


FACE SHIELD



ACID HOOD*

FACE PROTECTION



GOGGLES AND SPECTACLES

EYE PROTECTION

*Courtesy of Mine Safety Appliances Co.

information, consult American National Standards Institute Publication Z87.1-1968, Practice for Occupational and Educational Eye and Face Protection.

Utilization of a few simple common laboratory practices may avoid accidents that result in blindness. Supplies of caustic chemicals should be stored in the smallest size container compatible with daily need at the workbench. In the event of breakage or spill, this will minimize the hazard. Laboratory personnel handling chemicals that may be explosive, corrosive or caustic, or handling cryogenic materials, should be required to wear eye protection.

Personnel who normally wear corrective lenses and work in an area requiring eye protection must wear goggles or spectacles depending on the job assignment. The protective lenses of spectacles should provide optical correction. Goggles should fit over corrective spectacles without disturbing the adjustment of the spectacles or causing leakage, or incorporate corrective lenses mounted behind the protective goggles.

A basic rule to follow is that if an eye hazard exists for a particular operation or experiment, the soundest safety policy would be to require that eye or face protection, or both, be worn at all times by all persons entering or working in the laboratory. Safety glasses with metal or plastic frame spectacles, impact-resistant lenses, and side shields generally are adequate in most situations. Prescription safety lenses fabricated from ground and polished clear glass, or from plastics that may provide longer service life, are frequently required for laboratory personnel. The glass lenses are specially fabricated and heat treated so that they are resistant to impact. However, in those laboratories in which chemicals are used that may cause injury to the eye, it is necessary to use goggles, face shield, or perhaps a combination of them.

If eye protection is deemed necessary in a laboratory, then an emergency eyewash station should be available.

Contact lenses do not provide protection to the eyes. Foreign material present on the surface of the eye may become trapped in the

capillary space between the contact lenses and the cornea. Inert, but sharp, particles, caustic chemicals, irritating vapors, and infectious agents in this space cannot be washed off the surface of the cornea. If the material that gets into the eye is painful, it becomes extremely difficult to remove the contact lens because of the muscle spasms that may develop. In accordance with the position adopted by the National Society for the Prevention of Blindness, it is recommended that contact lenses not be worn around chemicals, fumes, and other hazardous materials and dust particles. The only exception is if a visual problem exists that is corrected only by contact lenses as certified by the employee's physician or optometrist. Where contact lenses are worn, eye protection, such as tight-fitting goggles, must be worn. The eye protective device used with the contact lenses must meet or exceed all the requirements of the American National Standards Institute as specified in Practice for Occupational and Educational Eye and Face Protection, Z87.1-1968.

4. Respiratory Protective Equipment

In recombinant DNA research, respiratory protection is required for emergency procedures and for work in a P4 facility suit area.

There are many kinds of respiratory protective devices from which to choose. They vary in design, application, and protective capability. They can be placed into three categories: air purifying, supplied air, and self-contained breathing apparatus.

a. Air-Purifying Respiratory Devices

These may contain both a mechanical filter and a chemical cartridge. The mechanical filter provides protection against biological aerosols. Mechanical filters consist of fibrous material that will remove the particles from air as it passes through the medium; however, they do not protect against harmful gases and vapors.

The chemical cartridge protects against specific gases and vapors present in the atmosphere not in excess of 0.1% by volume. Several types of cartridges are available from safety supply organizations. The type to be used for a particular operation is dependent upon the chemical protection required.

If the air-purifying devices are manufactured as "half-face masks," they protect only against entry through the nose and mouth. A "full-face protective mask" is a more sophisticated air-purifying device, that provides protection to the major portion of the face, eyes, and respiratory tract. It is more efficient in filtering out biological contaminants and removing gases and vapors.

Hospital or contagion-type masks are less efficient forms of air-purifying devices. Today, most of these masks are of the disposable type. Unfortunately, they do not permit a very good face seal. In addition, some exhibit low filtration efficiency.

The effectiveness of all air-purifying devices is dependent upon such factors as the resistance they present to breathing, their comfort when worn for long periods, and the effectiveness of the filter

material in removing particulates of a given size, the peripheral seal of the device, their design, and durability. This category of mask requires an efficient filtering medium and is dependent upon a good peripheral seal because without it the inhaled air will bypass the filter element and provide poor or no protection. A clean-shaven face is required if a mask or respirator is to provide a good face seal.

b. Supplied-Air Respiratory Devices

(1) Air Line Respirators

This device supplies air from a remote filtered source by pipe and hose line to a half- or full-face mask respirator. Air line respirators have been found most useful where maximum respiratory protection is required and where leakage through a filter or peripheral seal cannot be tolerated. The system does have the limitation that if the air supply fails the person using the respirator must leave the area immediately because the central system has a limited reserve air supply tank normally established at a 30-minute reserve. Another disadvantage is that the air supply hose limits the user to a certain fixed range from the air supply.

There are three broad categories of air line respirators: constant flow, demand flow, and pressure demand. Constant flow respirators are generally used under conditions of an ample air supply as supplied by compressors. Demand-type respirators are normally used where compressed air cylinders are available; however, for a laboratory engaged in hazardous activities, this type system is not suitable because of inward leakage caused by negative pressure during inhalation. The pressure-demand air line respirator provides a positive pressure during both inhalation and exhalation, and does not use as much air as the constant flow units.

(2) Air-Supplied Hoods

Air-supplied hoods, or complete suits, that obtain their source of air from an external filtered compressed air source have been used for those operations where it is impractical, or impossible, to isolate the product or hazardous operation in a protective cabinet or

other type enclosure. The air-supplied hoods would be used for those situations in which only a respiratory hazard is involved.

(3) Self-contained Apparatus

This apparatus operates independently of the surrounding atmosphere, since the mask comes with its own air supply. There are three basic types of self-contained breathing apparatus: oxygen cylinder re-breathing, demand, and self-generating. Normally, they may be used for only very short time periods, 15 to 30 minutes, because of the limited air supply available; however, the system is applicable when leakage through the filter or peripheral seal cannot be tolerated.

Self-contained systems are not used to any great extent in laboratories, except in case of emergencies. For those installations desiring to have a self-contained apparatus on hand in the event of an emergency, contact several of the reputable safety supply concerns and discuss with their technical personnel the various features of these devices before making a final selection.

c. Selecting a Respiratory Protective Device

The selection of what respiratory protective device to use should be made with knowledge of the conditions of the research activities and the risk situation involved. Selection should be made jointly by the principal investigator and safety officer. It should be emphasized that the degree of protection required must be thoroughly investigated; and, once determined, the respiratory equipment selected must be inspected and properly fitted. The reputable safety supply houses can provide data as to mask performance based on tests they have conducted or that they know have been performed by research institutions or government agencies referred to previously.

It cannot be overemphasized that there must be a good peripheral seal between the face and the mask. Such conditions as beard growth, temple pieces of eyeglasses, and the absence of one or both dentures all contribute to mask leakage. Full-face masks with prescription lenses

that do not interfere with the mask are available commercially, if required. When assigning a respirator to an individual, especially the half-face respirator, one size or type will not fit all subjects, and it is necessary to have two or more types and sizes of half-face masks available for fitting and use purposes. Determination that the proper tension exists on the respirator headband is also important, because it has been shown that a direct relationship exists between strap tightness and seal. Once the proper mask has been assigned to an individual, the next step is to ascertain that the wearer properly dons and adjusts the mask. Too frequently, adequate peripheral seal is not obtained because a mask is worn improperly.

d. Mask Decontamination

The newer type contagion or hospital-type masks are of the disposable category. If masks of this type have been worn in a contaminated area, autoclaving is recommended prior to discard. Where personnel have been working in an area that has resulted in overt contamination of the reusable respiratory protective equipment, ethylene oxide (ETO) must be used to assure complete penetration of the decontaminant. The facepiece, however, must be aerated 24 hours following decontamination because, if there is insufficient aeration, chemical burns can be inflicted on the user. This decontamination process will have an adverse effect on any charcoal filter element, and, therefore, any cartridge or canister that contains this adsorbent as a component of the overall mask should be replaced following sterilization. Autoclaving should not be used, as it has a deleterious effect on some of the compounds used to seal the filter material to the edge of the canister or cartridge.

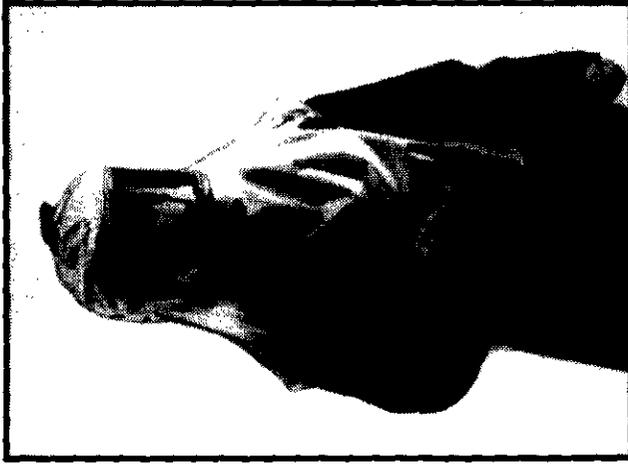
Personnel using respiratory protection devices should wipe down their equipment with a chemical disinfectant at the end of the day's activity. Several disinfectants may be used. A damp cloth that has been soaked in the disinfectant and the excess squeezed out should be used for the wipe down process of the facepiece. A hypochlorite solution (500 ppm)

with a wetting agent, or alcohol 85%, would be satisfactory. In any wipe down process, it is extremely important to reach all crevices. Following the wipe down procedure, the protective equipment should be thoroughly rinsed with clean, warm water and then exposed to free-flowing air at least 30 minutes before reuse. Valves, head straps, and other parts should be checked. Replace them with new parts, if defective. Insert new filters, cartridges, or canisters, if required; ascertain that the seal is tight. Place in plastic bag or container for storage.

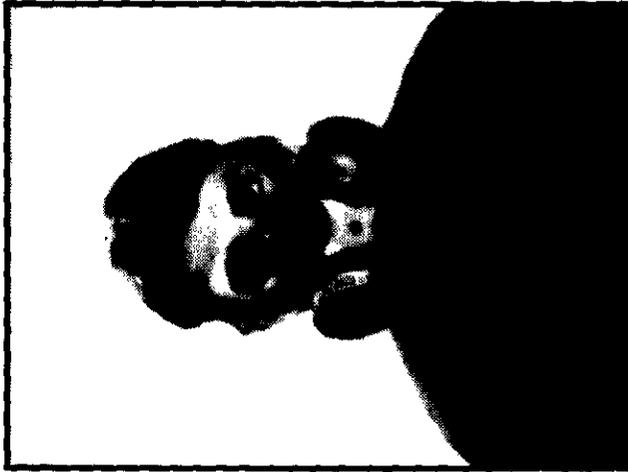
When applied frequently to equipment, several of the available disinfectants will cause corrosion of metal surfaces and require that parts of a mask be replaced from time to time.

For those situations in which personal hygiene is the only consideration, all rubber or plastic face masks and respirators should be scrubbed with a liquid detergent solution and decontaminated. Suitable disinfectants are the quaternary ammonium compounds (200 ppm in water with less than 500 ppm total hardness). Wipe off the decontaminated respiratory device with warm water to remove any residual quaternary compound remaining so as to avoid any possible dermatitis.

Following this decontamination procedure, half-face masks can be stored in plastic bags until required again. Full-face masks or other types of respirators should be stored in cartons or carrying cases, specifically fabricated for the protective equipment.



AIR SUPPLIED HEAD HOOD



HALF MASK FACEPIECE



FULL MASK FACEPIECE

RESPIRATORY PROTECTIVE EQUIPMENT

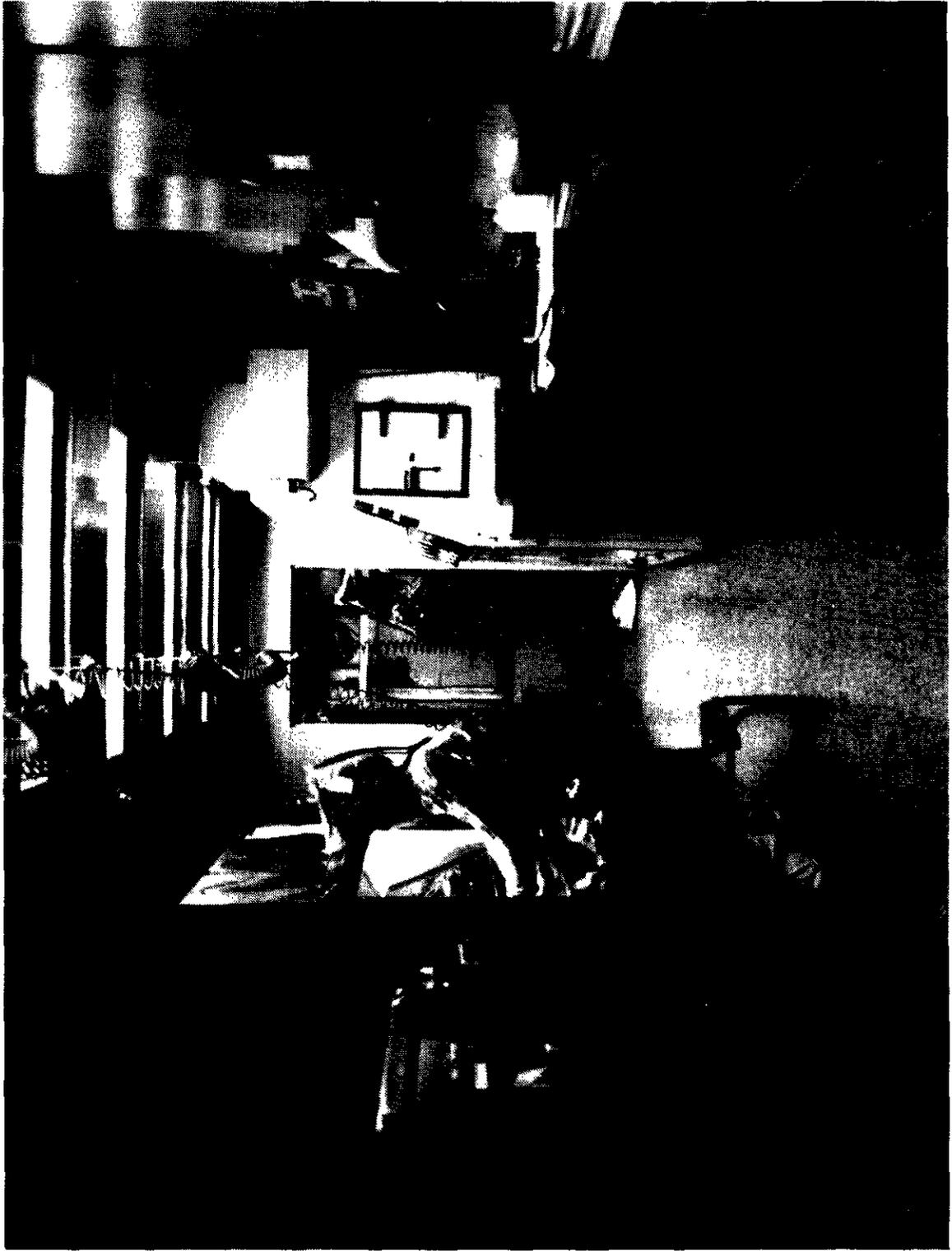
5. Positive Pressure Suits

One-piece positive pressure ventilated suits are required to be worn in all designated suit areas within P4 facilities.

Positive pressure suits are usually fabricated of heavy vinyl material. To provide the wearer with protection in the event the air supply is accidentally disconnected, a biological filter should be installed at the quick disconnect at the suit. This will provide the wearer protection to permit egress from the restricted zone.

Although they have been under development for several years, and many are in use in industry, there still are problems associated with their use. Personnel have found them to be cumbersome, some heat up rapidly in warm weather (especially the complete suits) unless conditioned air and a good air distribution system are provided within the protective garment, and many can be easily torn by sharp edges. Personnel with a good positive attitude adapt well to their use.

It is desirable to provide conditioned air and to include an air distribution system within the suit to permit the user to carry on activities in a comfortable environment. One air conditioning device utilizes a vortex tube that introduces either warm or cooled air. After use in a contaminated area, suits must be decontaminated by a thorough washdown with a liquid disinfectant. A 2% solution of peracetic acid is suitable and effective but requires special handling and equipment because it is quite corrosive and has an irritating odor.



ONE-PIECE POSITIVE PRESSURE VENTILATED SUITS

D. Housekeeping

Well-defined housekeeping procedures and schedules are essential in reducing the risks of working with biohazardous materials and in protecting the integrity of the research program. Housekeeping limits physical clutter, controls contamination, and facilitates the efficient use of chemical disinfectants. Although the term "housekeeping" can be broadly interpreted as including procedures such as decontamination, disposal, and animal care, the interpretation given here relates only to the concept of physical cleaning; those tasks universally considered to be janitorial in nature.

The objectives of housekeeping in the biological laboratory are to:

- provide an orderly and clean work area conducive to the accomplishment of the research program,
- provide work areas devoid of physical hazards,
- prevent the accumulation of materials from current and past experiments that constitute a hazard to laboratory personnel, and
- prevent the creation of aerosols of hazardous materials as a result of the housekeeping procedures used.

Procedures developed in the area of housekeeping should be based on the highest level of risk to which the personnel and integrity of the experiments will be subject. Such an approach avoids the confusion of multiple practices and retraining of personnel. The primary function, then, of routine housekeeping procedures is to prevent the accumulation of wastes that (i) may harbor microorganisms that are a potential threat to the integrity of the biological systems under investigation, (ii) may enhance the survival of microorganisms inadvertently released in experimental procedures, (iii) may retard penetration of disinfectants, (iv) may be transferable from one area to another on clothing and shoes, (v) may, with sufficient buildup, become a biohazard as a consequence of secondary aerosolization by personnel and air movement, and (vi) may cause allergic sensitization of personnel, e.g., to animal danders.

Housekeeping in animal care units has the same primary function as that stated for the laboratory and should, in addition, be as meticulously

carried out in quarantine and conditioning areas as in areas used to house experimentally infected animals. No other areas in the laboratory have the constant potential for creation of significant quantities of contaminated wastes than do animal care facilities.

In all laboratories, efforts to achieve total decontamination or to conduct a major cleanup are normally undertaken at relatively long time intervals. Routine housekeeping must be relied on to provide a work area free of significant sources of background contamination. The provision of such a work area is not simply a matter of indicating in a general way what has to be done, who will do it, and how often. The supervisor must view each task critically in terms of the potential biohazard involved, decide on a detailed procedure for its accomplishment, and provide instructions to laboratory personnel in a manner that minimizes the opportunity for misunderstanding.

The following checklist outlines a portion of the items requiring critical review by the laboratory supervisor. It is not intended to be complete, but is presented as an example of the detailed manner in which housekeeping in the biological laboratory complex must be viewed.

- Administration Areas
- Aisles
- Animal Food Storage
- Animal Bedding Storage
- Biological Safety Cabinets
- Bench Tops and Other Work Surfaces
- Ceilings
- Change Rooms
- Cleaning Solution Disposal
- Cages and Cage Racks
- Dry Ice Chests
- Deep Freeze Chests
- Entry and Exit Ways
- Equipment Storage
- Floors
- Glassware
- General Laboratory Equipment Cleanup
- Hallways
- Incubators
- Instruments
- Insect and Rodent Control

- Light Fixtures
- Mechanical Equipment Areas
- Mops
- Pipes - Wall and Ceiling Hung
- Refrigerators
- Showers
- Supply Storage
- UV Lamps
- Vacuum Cleaners
- Waste Accumulations
- Waste Water Disposal
- Others

Housekeeping in the laboratory is one of the avenues that leads to accomplishing the research program safely. It is important that housekeeping tasks be assigned to personnel who are knowledgeable of the research environment. The recommended approach to housekeeping is the assignment of housekeeping tasks to the research teams on an individual basis for their immediate work areas and on a cooperative basis for areas of common usage. Similarly, animal caretaker personnel should be responsible for housekeeping in animal care areas. The laboratory supervisor must determine the frequency with which the individual and cooperative housekeeping chores need be accomplished. He should provide schedules and perform frequent inspection to assure compliance. This approach assures that research work flow patterns will not be interrupted by an alien cleanup crew, delicate laboratory equipment will be handled only by those most knowledgeable of its particular requirements, and the location of concentrated biological preparations and contaminated equipment used in their preparation and application will be known.

1. Floor Care

Avoidance of dry sweeping and dusting will reduce the formation of nonspecific environmental aerosols. Wet mopping or vacuum cleaning with a high-efficiency particulate air (HEPA) filter on the exhaust is recommended.

Careful consideration must be given to design and quality in the selection of cleaning equipment and materials and in their use to prevent the substitution of one hazard for another.

In the absence of overt hazardous spills, the cleaning process commonly will consist of an initial vacuuming to remove all gross particulate matter and a follow-up wet mopping with a solution of chemical decontaminant containing a detergent. Depending on the nature of the surfaces to be cleaned and availability of floor drains, removal of residual cleaning solutions can be accomplished by a number of methods. Among these are: pickup with a partially dry mop, pickup with a wet vacuum that has an adequately filtered exhaust, or removal to a convenient floor drain by use of a floor squeegee.

2. Dry Sweeping

While it is recommended that dry sweeping be minimized, this may be the only method available or practicable under certain circumstances. In such cases, sweeping compounds used with push brooms and dry-dust mopheads treated to suppress aerosolization of dust should be used.

Sweeping compounds available from the usual janitorial supply firms fall in three categories:

- wax-based compounds used on vinyl floors and waxed floor coverings
- oil-based compounds for concrete floors
- oil-based compounds with abrasives (such as sand) to achieve a dry scouring action where much soil is present

Dry-dust mopheads can be purchased as treated disposable units or as reusable, washable heads that must be treated with appropriate sprays or by other means to improve their dust-capturing property.

3. Vacuum Cleaning

In the absence of a HEPA filter on the exhaust, the usual wet and dry industrial-type vacuum cleaner is a potent aerosol generator. The HEPA-filtered exhaust used in conjunction with a well-sealed vacuum unit, however, can negate this factor because of its ability to pass large volumes of exhaust air while retaining particles with a minimum efficiency of 99.97%. Wet and dry units incorporating a HEPA filter on the exhaust are available from a number of manufacturers. The filter in its housing should provide the rated efficiency.

There are no particular requirements with respect to the manner in which the dry vacuuming is accomplished other than to emphasize that the objective is to remove all debris and particulate matter. The manufacturer's directions adequately detail the frequency of bag changes, filter changes, and mechanical adjustments.

Dry material vacuum-collected during these floor-cleaning activities is potentially contaminated, but the nature of the risk is probably greater to the experiment than to the experimenter. It is wise to effect bag and filter changes and to clean out collection tanks in a manner that will avoid or minimize aerosolizing the contents of the vacuum cleaner.

A vacuum machine that collects debris in a disposable bag is preferable to machines that collect the major debris in a tank and on an exposed primary filter. Even though it may serve as a primary filter, the disposable bag must be removed with caution. A bellows effect may pump dust out of the bag if its intake opening is not sealed before moving it to a plastic bag for transfer out of the area. In any event, the outer surface of the disposable bag will probably bear some dust contamination, which also may occur on inner surfaces of the machine.

To avoid contaminating experimental materials, the emptying of vacuum collection tanks and changing of bags and filters are best done away from the immediate laboratory area, for example, in a small area that can be easily cleaned afterwards. The use of heavy rubber gloves is recommended when removing wastes from tanks in case broken glass is present. After

making the filter changes, all external surfaces of the immediate work area and the equipment should be wiped with a cloth moistened in decontaminant. The operator might plan for a change of laboratory clothing afterwards so as to minimize carrying contamination into other areas of the laboratory.

Avoid use of dry vacuum cleaning equipment in work with high risk agents in the open laboratory. Should it be necessary to use it, it is recommended that gaseous sterilization be used to minimize aerosolization of microorganisms before waste is emptied from the vacuum container. Because complete penetration of sterilizing gases into the collected dry dust may be a problem, all wastes should be placed in a plastic bag, which then is tightly closed and incinerated or disposed of in an approved manner.

When dry vacuum cleaning equipment has been used within a gastight safety cabinet system, it can be treated in an attached double-door carboxyclave (an autoclave equipped with an ethylene oxide gas sterilization system) to allow for removal and emptying of the collection tank.

If a wet vacuum is to be used for pickup of the detergent-germicide solution from the floor, the manufacturer's recommendations on filter life should be followed. In addition, the operation of the vacuum should be closely observed for evidence of operating changes indicating restricted airflow or, conversely, increased flow indicating filter failure. Liquids collected in the vacuum cleaner after floor mopping will contain disinfectant material. These liquids may be poured down a convenient floor drain, except in the case of cleanup wastes from an overt spill. The collected liquid should then be autoclaved or treated with chlorine solution before disposal.

Provisions should be made for regular decontamination of the entire vacuum cleaner with formaldehyde gas or vapor, or ethylene oxide. This should be done after use if the vacuum is used in any manner for cleanup of overt spills of infectious material.

4. Selection of a Cleaning Solution

The selection of a detergent-disinfectant combination for routine cleaning of the laboratory complex should be based on the requirements of the area of greatest potential for contamination by the widest spectrum of microorganisms. With rare exception, this will be identified as the animal holding area and the expected microorganisms may well include fungi, viruses, and the vegetative and spore forms of bacteria. A disinfectant solution for such a range of microorganisms would, however, be expensive and excessively corrosive for routine use. Except in those rare instances where it can be assumed that pathogenic spores are being shed by laboratory animals, the risks from the spores are more likely to affect the experiments than the personnel. The spores tend to be associated with organic debris from bedding and food, thus offering potential for removal or at least a large initial reduction in their numbers by vacuum cleaning. A wide range of cleaning solutions that are mildly sporicidal, reasonably residual, and are not destructive to the physical plant is available. Phenol derivatives in combination with a detergent have these characteristics and have been selected for routine use in a number of research facilities. There are numerous detergent-phenolic combinations available on the market. The phenols are one type of a broad spectrum of biocidal substances that include the mercurials, quaternary ammonium compounds, chlorine compounds, iodophors, alcohols, formaldehyde, glutaraldehyde, and combinations of alcohol with either iodine or formaldehyde. These have been discussed in Section II,E.

The laboratory supervisor should make a selection from the types most readily available that meet the general criteria of effectiveness, residual properties, and low corrosiveness.

5. Wet Mopping - Two-Bucket Method

Wet mopping of floors in laboratory and animal care areas is, from a safety standpoint, most conveniently and efficiently accomplished using a two-bucket system. The principal feature of such a system is that fresh detergent-decontaminant solution is always applied to the floor from one bucket, while all spent cleaning solution wrung from the mop is collected in the second bucket. Compact dolly-mounted double-bucket units with foot-operated wringers are available from most janitorial supply houses. A freshly laundered mophead of the cotton string type should be used daily. This requires that a mop with removable head be provided as opposed to a fixed-head type. In practice, the mop is saturated with fresh solution, very lightly wrung into the second bucket and applied to the floor using a figure eight motion of the mophead. After every four or five strokes, the mophead is turned over and the process continued until an area of approximately 100 ft² has been covered. After allowing a contact time of five minutes, the solution is removed with either a wet vacuum cleaner with HEPA-filtered exhaust or with the wrung-out mop. The mopping is continued in 100 ft² increments until the total floor area has been covered. Floor-cleaning procedures are most effectively completed after the majority of the work force has departed and should progress from areas of least potential contamination to those of greatest potential. Before a mophead is sent to a laundry, it should be autoclaved. Spent cleaning fluids are disposed of by flushing down the drain.

If the cleanup follows an overt spill of infectious material, the spent cleaning solution, after removal from the floor, should be autoclaved or treated with chlorine solution. Chlorine (as household bleach) should be added to give 500 ppm and held for a contact time of 15 minutes before dumping in the sanitary sewer.

6. Alternative Floor-Cleaning Method for Animal Care Areas and Areas with Monolithic Floors

The absence of permanently placed laboratory benches and fixed equipment, coupled with the mobility of modern cage racks, makes possible alternate floor-cleaning procedures in animal care facilities. As in all considerations of methodologies in biomedical laboratory facilities, it is necessary to assess the compatibility of procedures and facilities from the hazard point of view. The alternative floor-cleaning procedure to be discussed requires that floors are completely sealed or of monolithic construction so that liquid leakage to adjacent areas does not occur and that floor drains or wet vacuum cleaners are available.

Subsequent to the removal of all debris by dry vacuum, move the cage racks to one side of the room. Cover the floor of the remaining cleared portion of the room with detergent-disinfectant solution applied at a rate of approximately one gallon per 144 ft² from a one-gallon tank sprayer, using a setting of the nozzle that will cause the solution to flow on and not create a spray. The nozzle is placed close to the floor. Allow a fifteen-minute contact period; then push the cleaning solution to the floor drain with a large floor squeegee or pick it up with a wet vacuum. Allow the floor to air dry; move the cage racks into the cleaned area, and repeat the process for the remaining floor area. Floor drains in these areas should be rim-flush, at least six inches in diameter, and fitted with a screen or porous trap bucket to catch large debris that escapes the initial dry cleaning. Such screens and baskets should be emptied after treatment with a disinfectant. If space utilization does not require frequent floor washdown, pour a half-gallon of detergent-disinfectant solution into the drain each week to keep the trap in the waste line filled against backup of sewer gases.

E. Decontamination and Disposal

Historical data on the efficacy of various antimicrobial chemicals indicate that no major surprises will be forthcoming regarding the susceptibility of organisms containing recombinant DNA molecules. In the absence of adequate information, tests to determine the efficacy of candidate disinfectants should be conducted with the specific agent of interest. The goal of disinfection is not only the protection of personnel and the environment from exposure to biological agents, but also the prevention of contamination of experimental materials by the ubiquitous background of microorganisms. This additional factor should be considered in selecting germicidal materials and methods.

1. Disinfectant Methods

Physical and chemical means of disinfection fall into four main categories: Heat, Liquid Disinfectants, Vapors and Gases, and Radiation.

a. Heat

The application of heat, either moist or dry, is recommended as the most effective method of sterilization. Steam at 121°C under pressure in the autoclave is the most convenient method of rapidly achieving sterility. Dry heat at 160° to 170°C for periods of 2 to 4 hours is suitable for destruction of viable agents on impermeable nonorganic material such as glass, but is not reliable in even thin layers of organic or inorganic material that can act as insulation. Incineration kills microorganisms and serves as an efficient means for disposal.

b. Liquid Disinfectants

In general, the liquid disinfectants find their most practical use in surface treatment and, at sufficient concentration, as sterilants of liquid waste for final disposal in sanitary sewerage systems.

There are many misconceptions concerning the use of liquid disinfectants. This is due largely to a characteristic capacity of such liquids to perform dramatically in the test tube and to fail miserably in a practical situation. Such failures often occur because proper consideration was not given to such factors as temperature, time of contact, pH, concentration, and the presence and state of dispersion, penetrability and reactivity of organic material at the site of application. Small variations in the above factors may make large differences in effectiveness of disinfection. For this reason, even when used under highly favorable conditions, complete reliance should not be placed on liquid disinfectants when the end result must be sterility.

There are many liquid disinfectants available under a wide variety of trade names. In general, these can be categorized as halogens,

acids or alkalies, heavy metal salts, quaternary ammonium compounds, phenolic compounds, aldehydes, ketones, alcohols and amines. Unfortunately, the more active disinfectants often possess undesirable characteristics, such as corrosive properties. None is equally useful or effective under all conditions.

c. Vapors and Gases

A variety of vapors and gases possess germicidal properties. The most useful of these are formaldehyde and ethylene oxide. When these can be employed in closed systems and under controlled conditions of temperature and humidity, sterilization can be achieved. Vapor and gas disinfectants are primarily useful in sterilizing: (i) Biological Safety Cabinets and associated effluent air-handling systems and air filters; (ii) bulky or stationary equipment that resist penetration by liquid surface disinfectants; (iii) instruments and optics that might be damaged by other sterilization methods; and (iv) rooms and buildings and associated air-handling systems.

d. Radiation

Ionizing radiation will destroy microorganisms. The germicidal action of X-rays has been known for 80 years. Gamma radiation is used for the destruction of microorganisms in some food products and for the sterilization of certain medical products. Ionizing radiation is not a practical tool for laboratory use. However, ultraviolet radiation (UV) is a practical method for inactivating viruses, mycoplasma, bacteria and fungi. This nonionizing radiation is especially useful for the destruction of airborne microorganisms and, to a lesser extent, for the inactivation of microorganisms on exposed surfaces or for the treatment of products of unstable composition that cannot be treated by conventional methods. The usefulness of ultraviolet radiation as a sanitizer is limited by its low penetrating power. Information is not available regarding the effectiveness of UV irradiation for inactivating microorganisms containing recombinant DNA molecules, but it is highly unlikely that increased resistance to UV is

imparted to a cell by the insertion of recombinant DNA. Ultraviolet light is primarily useful in air locks, animal holding areas, ventilated cabinets and in laboratory rooms during periods of nonoccupancy to reduce the levels of viable airborne microorganisms and to maintain good air hygiene.

2. Characteristics of Chemical Disinfectants in Common Use in Laboratory Operations

Those persons working with viable microorganisms will find it necessary to disinfect work areas and materials, equipment, and specialized instruments by chemical methods. Chemical disinfection is necessary because the use of pressurized steam, the most reliable method of sterilization, is not normally feasible for disinfecting large spaces, surfaces, and stationary equipment. Moreover, high temperatures and moisture often damage delicate instruments, particularly those having complex optical and electronic components.

Chemical disinfectants are available as powders, crystals, liquid concentrates or compressed gases. Use concentrations must be determined and dilutions made as required. Chemical disinfectants that are gaseous at room temperature may be useful as space disinfectants. Others become gases at reasonably elevated temperatures and can act as either aqueous surface or gaseous space disinfectants.

Inactivation of microorganisms by chemical disinfectants may occur in one or more of the following ways: (i) coagulation and denaturation of protein, (ii) lysis, (iii) inactivation of an essential enzyme by either oxidation, binding, or destruction of enzyme substrate. The relative resistance to the action of chemical disinfectants can be substantially altered by such factors as: concentration of active ingredient, duration of contact, pH, temperature, humidity, and presence of organic matter. Depending upon how these factors are manipulated, the degree of success achieved with chemical disinfectants may range from minimal inactivation of target microorganisms to sterility within the limits of sensitivity of the assay systems employed.

There are dozens of disinfectants available under a wide variety of trade names. In general, these disinfectants can be classified as acids or alkalies, halogens, heavy metal salts, quaternary ammonium compounds, phenolic compounds, aldehydes, ketones, alcohols, and amines. Unfortunately, the more active the disinfectant, the more likely it will possess undesirable characteristics. For example, peracetic acid is a fast-acting, universal

germicide. However, in the concentrated state it is a hazardous compound that can readily decompose with explosive violence. When diluted for use, it has a short half-life, produces strong, pungent, irritating odors, and is extremely corrosive to metals. Nevertheless, it is such an outstanding germicide that it is commonly used in germ-free animal studies despite these undesirable characteristics.

The halogens are a most active group of disinfectants. Chlorine, iodine, bromide, and fluorine will rapidly kill bacterial spores, viruses, rickettsiae, and fungi. Free halogen is the effective agent. These disinfectants are effective over a wide range of temperatures. The halogens have several undesirable features. They combine readily with protein, so that an excess of the halogen must be used if proteins are present. Also, the halogens are somewhat unstable, especially at lower pH levels, so that fresh solutions must be regularly prepared. Finally, the halogens corrode metals. A number of manufacturers of disinfectants have treated the halogens to control some of these undesirable features. For example, sodium hypochlorite reacts with p-toluenesulfonamide to form Chloramine T, and iodine reacts with certain surface-active agents to form the popular iodophors. These "tamed" halogens are relatively stable, nontoxic, odorless, and less corrosive to metals. The buffering of these compounds, however, decreases their germicidal effectiveness. This trade-off is required when these compounds are used in metal pans or dunk tanks.

Ineffectiveness of a disinfectant is often due to the failure of the disinfectant to contact the microorganism rather than failure of the disinfectant to act. If one places an item in a liquid disinfectant, one can see that the item is covered with tiny bubbles. Of course, the area under the bubbles is dry, and microorganisms in these dry areas will not be affected by the disinfectant. Also, if there are spots of grease, rust or dirt on the object, microorganisms under these protective coatings will not be contacted by the disinfectant. Scrubbing an item when immersed in a disinfectant is helpful, and a disinfectant should have, and most do have, incorporated surface-active agents.

3. Properties of Some Common Disinfectants

a. Alcohol

Ethyl or isopropyl alcohol in a concentration of 70-85% by weight is often used. Alcohols denature proteins and are somewhat slow in their germicidal action. However, they are effective disinfectants against lipid-containing viruses.

b. Formaldehyde

Formaldehyde for use as a disinfectant is usually marketed at about 37% concentration of the gas in water solution referred to as formalin or as a solid polymerized compound called paraformaldehyde. Formaldehyde in a concentration of 5% active ingredient is an effective liquid disinfectant. Formaldehyde at 0.2 to 0.4% is often used to inactivate viruses in the preparation of vaccines. Formaldehyde loses considerable disinfectant activity at refrigeration temperatures. Its pungent, irritating odor requires that care be taken when using formaldehyde solutions in the laboratory. Formaldehyde vapor generated from formaldehyde solution is an effective space disinfectant for sterilizing rooms or buildings. Formaldehyde gas can be generated by heating paraformaldehyde to depolymerize it. In the absence of high moisture content in the air, formaldehyde released in the gaseous state forms less polymerized residues on surfaces and less time is required to clear treated areas of fumes than formaldehyde released in the vapor state.

c. Phenol

Phenol itself is not often used as a disinfectant. The odor is somewhat unpleasant and a sticky, gummy residue remains on treated surfaces. This is especially true during steam sterilization. Although phenol itself may not be in widespread use, phenol homologs and phenolic compounds are basic to a number of popular disinfectants. The phenolic compounds are effective disinfectants against some viruses, rickettsiae, fungi and vegetative bacteria. The phenolics are not effective in ordinary usage against bacterial spores.

d. Quaternary Ammonium Compounds or Quats

After 40 years of testing and use, there is still considerable controversy about the efficacy of the "Quats" as disinfectants. These cationic detergents are strongly surface-active and this detergency property makes them good surface cleaners. The Quats will attach to protein so that dilute solutions of Quats will lose effectiveness in the presence of proteins. The Quats tend to clump microorganisms and are neutralized by anionic detergents, such as soap. The Quats are bacteriostatic, tuberculostatic, sporostatic, fungistatic and algistatic at low concentrations. They are bactericidal, fungicidal, algicidal and virucidal against lipophilic viruses at medium concentrations, but they are not tuberculocidal, sporicidal or virucidal against hydrophilic viruses even at high concentrations. The Quats have the advantages of being odorless, nonstaining, noncorrosive to metals, stable, inexpensive and relatively nontoxic.

e. Chlorine

This halogen is a universal disinfectant active against all microorganisms, including bacterial spores. Chlorine combines with protein and rapidly decreases in concentration in its presence. Free, available chlorine is an active element. It is a strong oxidizing agent, corrosive to metals. Chlorine solutions will gradually lose strength so that fresh solutions must be prepared frequently. Sodium hypochlorite is usually used as a base for chlorine disinfectants. An excellent disinfectant can be prepared from household or laundry bleach. These bleaches usually contain 5.25 percent available chlorine or 52,500 ppm. If one dilutes them 1 to 100, the solution will contain 525 ppm of available chlorine; and if a nonionic detergent is added in a concentration of about 0.7 percent, a very good disinfectant is created.

f. Iodine

The characteristics of chlorine and iodine are similar. One of the most popular groups of disinfectants used in the laboratory is the

iodophors, and Wescodyne is perhaps the most widely used. The range of dilution of Wescodyne recommended by the manufacturer is 1 oz. in 5 gal. of water giving 25 ppm of available iodine to 3 oz. in 5 gal. giving 75 ppm. At 75 ppm, the concentration of free iodine is .0075 percent. This small amount can be rapidly taken up by extraneous protein present. Clean surfaces or clear water can be effectively treated by 75 ppm available iodine, but difficulties may be experienced if any appreciable amount of protein is present. For washing the hands or for use as a sporicide, it is recommended that Wescodyne be diluted 1 to 10 or 10% in 50% ethyl alcohol, which will give 1,600 ppm of available iodine at which concentration relatively rapid inactivation of any and all microorganisms will occur.

4. Vapors and Gases for Space Decontamination

The use of formaldehyde as a vapor or gas has already been discussed. Other chemical disinfectants that have been used as space decontaminants include ethylene oxide, peracetic acid, beta-propiolactone (BPL), methyl bromide, and glutaraldehyde. When these can be used in closed systems and under controlled conditions of temperature and humidity, excellent disinfection can be obtained. Ethylene oxide adsorbed by materials such as rubber must be removed by aeration; otherwise, ethylene oxide is convenient to use, versatile, and noncorrosive. Peracetic acid is corrosive for metals and rubber. BPL is not recommended as a space disinfectant, since it is listed as a carcinogen by OSHA (Federal Register, Part III, Vol. 39, No. 20, January 29, 1974).

Formaldehyde is, in general, the chemical of choice for space disinfection. Safety cabinets, incubators, refrigerators, laboratory rooms, buildings, or other enclosed spaces can be disinfected with formaldehyde. The formaldehyde can be generated from aqueous solutions (formalin) containing 37-40% formaldehyde by heating or by vaporizing the solution. Formaldehyde gas, also, can be generated by heating paraformaldehyde, which is a solid polymer that contains 91 to 99% formaldehyde. If aqueous formaldehyde is used, the application rate should be one milliliter for each cubic foot of space to be treated. Also, if a small amount of exhaust air is used to keep the area being treated under a slightly reduced pressure, then this amount must be known, and one milliliter of formalin added for each cubic foot of exhaust air for at least a one-hour period. To assure thorough mixing, the use of air-circulating fans may be required. Areas being treated should have a temperature of at least 70°F (21°C) and a relative humidity of above 70%. Spaces being treated should not be wet, have condensate on the walls, or have pools of water on the floor, since formaldehyde is quite soluble in water and will be rapidly taken up. Also, as the water evaporates, polymerization will take place on the surfaces and these polymers are difficult to remove. Formaldehyde is a powerful reducing agent and is noncorrosive to metals. It can normally be assumed that any equipment or apparatus that will not be damaged by the humidity

necessary for decontamination will not be damaged by the formaldehyde. Although formaldehyde will sterilize all exposed surfaces, it has limited penetrating abilities, and materials that are tightly covered may not be sterilized. This lack of penetrating power is often an advantage in using formaldehyde, since the space need only be enclosed relatively tightly, and not hermetically sealed -- a condition impossible to achieve when rooms or buildings are being treated.

Generally, the generation of formaldehyde gas from powdered or flake paraformaldehyde by heating is the preferred method. Paraformaldehyde will depolymerize and convert to the gaseous state when heated to a temperature above 150°C. There are various practical methods for heating the paraformaldehyde to above 150°C., but the commercially available electric frying pan equipped with a thermostat is one of the simplest. The electric cord of the frying pan should be equipped with a one-hour timer so that the pan can be placed in the space to be treated and, after the submission of the formaldehyde gas, the power to the frying pan will be turned off automatically. The frying pan can hold one kilogram of flake formaldehyde. The depolymerization rate of paraformaldehyde is about 20 g per minute when the thermostat is set at 232°C. A concentration of 0.3 g of paraformaldehyde for each cubic foot of space to be treated is employed. Temperature of the space must be above 20°C and relative humidity 70% or higher. Exposure times should be at least two hours and, if possible, the exposure should be for eight hours or overnight. Formaldehyde generated from paraformaldehyde has better penetration, and fewer problems with condensation and subsequent need for prolonged aeration, than with formaldehyde generated from formalin. If walls and surfaces were not wet with condensation during the formaldehyde treatment process, then aeration and removal of the formaldehyde should proceed rapidly. A small room with nonporous surfaces and no materials or equipment in the room can be cleared of all detectable formaldehyde in less than an hour of aeration. However, an entire building containing a variety of surfaces and equipment may take many hours or even a day or more of aeration to remove the formaldehyde.

Formaldehyde is a toxic substance having a threshold limit value (TLV) of 2 ppm. Considerable caution must be exercised in handling, storing and using formaldehyde. Repeated exposure to formaldehyde is known to produce a hypersensitive condition in certain individuals. Self-contained breathing apparatus, air-supplied masks or industrial-type gas masks should be available and used whenever exposure to formaldehyde is possible. Most individuals can readily detect formaldehyde in a concentration of 1 ppm, which serves as a warning to avoid excessive exposure. Chemicals, such as anhydrous ammonia, have been used to neutralize formaldehyde and deposited paraformaldehyde with limited success. Air containing formaldehyde can be passed through alumina to adsorb the formaldehyde. This technique is useful in removing formaldehyde from cabinets and other small places, but impractical quantities of alumina are required for removing the formaldehyde from large rooms or buildings. Recent reports indicate that formaldehyde may combine with hydrochloric acid to form bis(chloromethyl) ether, a compound which is carcinogenic. When formaldehyde is to be used as a space disinfectant, the area to be treated should be surveyed to insure that there are no open containers of any acidic solution containing chloride ion. It should be mentioned that formaldehyde in the concentrations used for space disinfection has no effect on cockroaches nor possibly on other insects or arachnids as well.

Formaldehyde is explosive at concentrations between 7.0 and 73.0% by volume in air. This concentration, however, cannot be reached when standard procedures are used.

5. Residual Action of Disinfectants

As noted in the preceding discussion of disinfectant properties, some of the chemical disinfectants have residual properties that may be considered a desirable feature in terms of aiding in the control of background contamination. One is cautioned, however, to consider residual properties carefully. Ethylene oxide used to sterilize rubber products may be adsorbed by the rubber and desorbed slowly. Therefore, if the rubber products (shoes, gloves, respirators) are not thoroughly aerated (e.g., at least 24 hours), the ethylene oxide leaving the rubber material that is in contact with the skin may cause severe skin irritation. Cell cultures, as well as viruses of interest, may be inhibited or inactivated by disinfectants persisting after routine cleaning procedures. Therefore, reusable items that are routinely held in a liquid disinfectant prior to autoclaving and cleaning should receive particular attention in rinse cycles. Similarly, during general area sterilization with gases or vapors, it may be necessary to protect new and used clean items such as glassware, by removing them from the area or by enclosing them in gastight bags or by insuring adequate aeration following sterilization.

6. Laboratory Spills

A problem that may occur in the laboratory is an overt biological spill. A spill that occurs in the open laboratory may create a serious problem. Every effort should be taken to avoid such occurrences. A spill poses less of a problem if it occurs in a Biological Safety Cabinet provided splattering to the outside of the cabinet does not occur. Direct application of concentrated liquid disinfectant and a thorough wipe down of the internal surfaces of such cabinetry will usually be effective for decontaminating the work zone, but gaseous sterilants will be required to disinfect the interior sections of the cabinet. Each researcher must realize that in the event of an overt accident, research materials such as tissue cultures, media, and animals within such cabinets may well be lost to the experiment.

a. Spill in a Biological Safety Cabinet

A spill that is confined to the interior of the Biological Safety Cabinet should present little or no hazard to personnel in the area. However, chemical disinfection procedures should be initiated at once while the cabinet ventilation system continues to operate to prevent escape of contaminants from the cabinet. Spray or wipe walls, work surfaces, and equipment with a disinfectant. A disinfectant with a detergent has the advantage of detergent activity, which will help clean the surfaces by removing both dirt and microorganisms. A suitable disinfectant is a 3% solution of an iodophor such as Wescodyne or a 1 to 100 dilution of a household bleach (e.g. Clorox) with 0.7% nonionic detergent. The operator should wear gloves during this procedure. Use sufficient disinfectant solution to ensure that the drain pans and catch basins below the work surface contain the disinfectant. Lift the front exhaust grill and tray and wipe all surfaces. Wipe the catch basin and drain the disinfectant into a container. This disinfectant, gloves, wiping cloth and sponges should be discarded into an autoclave pan and autoclaved. This procedure will not disinfect the filters, blower, air ducts or other

interior parts of the cabinet. If the entire interior of the cabinet is to be sterilized, then this can be accomplished by the formaldehyde gas method using powdered or flake paraformaldehyde. Calculate the volume of the cabinet in cubic feet and weigh out 0.3 g of flake paraformaldehyde for each cubic foot of space. Place the paraformaldehyde in the frying pan and place the pan in the cabinet with the electric line run to the outside of the cabinet. Raise the humidity within the cabinet to about 70%. Vaporization of water in the frying pan is a convenient technique. Set the thermostat of the frying pan containing the paraformaldehyde at 450° F. Seal the cabinet opening with sheet plastic and tape. If the cabinet exhaust air is discharged into the room, attach flex hose to the cabinet exhaust port and extend the hose to the room exhaust grille; however, if the building exhaust air recirculates, attach flex hose to an open window or door. If the cabinet is exhausted directly into the building system, close the exhaust damper. Plug in the frying pan to depolymerize the paraformaldehyde. After one-half volume of paraformaldehyde has been depolymerized, turn on the cabinet fan for about three seconds to allow the formaldehyde gas to reach all areas. After depolymerization is complete, again turn on the cabinet fan for three seconds. Then allow the cabinet to stand for a minimum of one hour. After the one-hour exposure, open the flex hose on the exhaust damper, slit the plastic covering the opening and turn on the cabinet fan. Ventilate the cabinet for several hours to remove all traces of formaldehyde.

b. Spill in the Open Laboratory

If potentially hazardous biological material is spilled in the laboratory, the first essential is to avoid inhaling any airborne material by holding the breath and leaving the laboratory. Warn others in the area and go directly to a wash or change room area. If clothing is known or suspected to be contaminated, remove the clothing with care, folding the contaminated area inward. Discard the clothing into a bag or place the clothing directly in an autoclave. Wash all potentially contaminated areas

as well as the arms, face and hands. Shower if facilities are available. Reentry into the laboratory should be delayed for a period of 30 minutes to allow reduction of the aerosol generated by the spill. Advance preparation for management of a spill is essential. A "spill kit," including leak-proof containers, forceps, paper towels, sponges, disinfectant, respirators, and rubber gloves, should be readily available. A high-intensity, portable ultraviolet lamp is useful in emergency situations. This UV lamp can be moved into the room where the accident occurred and the automatic timer set for a given period of exposure. A delay timer allows sufficient time to get out of the room before the UV lamp is automatically activated. The door to the room should be locked or a sign posted on the door warning personnel not to enter as 1200 watts of radiation is emitted by this lamp. A 2-3 hour exposure will sterilize microorganisms that either may be airborne or have settled on exposed surfaces. Radiant energy at 253.7μ has little penetrating power so that microorganisms covered with dirt or dust will probably not be affected.

Protective clothing should be worn when entering the laboratory to clean the spill area. Rubber gloves, autoclavable footwear, an outer garment and a respirator should be worn. If the spill was on the floor, do not use a surgical gown that may trail on the floor when bending down. Take the "spill kit" into the laboratory room, place a discard container near the spill, and transfer large fragments of material into it; replace the cover. Using a hypochlorite containing 1,000 ppm available chlorine, iodophor solution containing 1,600 ppm iodine, or other appropriate disinfectant, carefully pour the disinfectant around and into the visible spill. Avoid splashing. Allow 15 minutes' contact time. Use paper or cloth towels to wipe up the disinfectant and spill, working toward the center of the spill. Discard towels into a discard container as they are used. Wipe the outside of the discard containers, especially the bottom, with a towel soaked in a disinfectant. Place the discard container and other materials in an autoclave and sterilize. Remove shoes, outer clothing, respirator and gloves and sterilize by autoclaving or exposure to ethylene oxide. Wash hands, arms and face or, if available, shower. If gaseous

disinfection of the laboratory room is to be carried out, follow the procedures as outlined in Section II. E. 4.

c. Radioactive Biohazard Spill Outside a Biological Safety Cabinet

In the event that a biohazardous spill also involves a radiation hazard, the cleanup procedure may have to be modified, depending on an evaluation of the risk assessment of relative biological and radiological hazard.

Laboratories handling radioactive substances will have the services of the designated radiation area supervisor to aid in the cleanup. Before cleanup procedures begin, a radiation protection officer should survey the spill for external radiation hazard to determine the degree of risk. In most cases, the spill will involve ^{14}C or ^3H , which present no external hazard. However, if more energetic beta or gamma emitters are involved, care must be taken to prevent hand and body radiation exposure. The radiation protection officer must make this determination before the cleanup operation is begun.

If the radiation protection officer approves, the biohazard handling procedure may begin: Using an autoclavable dust pan and squeegee, transfer all contaminated materials (paper towels, glass, liquid, gloves, etc.) into a deep autoclave pan. Cover the pan with aluminum foil or other suitable cover and autoclave according to standard directions.

If the radiation protection officer determines that radioactive vapors may be released and thereby contaminate the autoclave, the material must not be autoclaved. In that case, sufficient disinfectant solution to immerse the contents should be added to the waste container. The cover should be sealed with waterproof tape, and the container stored and handled for disposal as radioactive waste. Radioactive and biohazard warning symbols should be affixed to the waste container. As a general rule, autoclaving should be avoided. A final radioactive survey should be made of the spill area, cleanup tools, and shoes and clothing of individuals who had been in the area by taking swipes and counting in an appropriate counter.

7. Disposal

Decontamination and disposal in infectious disease laboratories are closely interrelated acts in which disinfection constitutes the first phase of disposal. All materials and equipment used in research on recombinant DNA molecules will ultimately be disposed of; however, in the sense of daily use, only a portion of these will require actual removal from the laboratory complex or on-site destruction. The remainder will be recycled for use either within the same laboratory or in other laboratories that may or may not engage in recombinant DNA research. Examples of the latter are: reusable laboratory glassware, instruments used in necropsy of infected animals, and laboratory clothing. Disposal should therefore be interpreted in the broadest sense of the word, rather than in the restrictive sense of dealing solely with a destructive process.

The principal questions to be answered prior to disposal of any objects or materials from laboratories dealing with potentially infectious microorganisms or animal tissues are:

- Have the objects or materials been effectively disinfected or sterilized by an approved procedure?
- If not, have the objects or materials been packaged in an approved manner for immediate on-site incineration or transfer to another laboratory?
- Does disposal of the disinfected or sterilized objects or materials involve any additional potential hazards, biological or otherwise, to those carrying out the immediate disposal procedures or those who might come into contact with the objects or materials outside the laboratory complex?

Laboratory materials requiring disposal will normally occur as liquid, solid, and animal room wastes. The volume of these can become a major problem when there is the requirement that all wastes be disinfected prior to disposal. It is most evident that a significant portion of this problem can be eliminated if the kinds of materials initially entering the laboratory are reduced. In any case, and wherever possible, materials not essential to the research should be retained in the nonresearch areas for

disposal by conventional methods. Examples are the packaging materials in which goods are delivered, disposable carton-cages for transport of animals, and large carboys or tanks of fluids that can be left outside and drawn from as required. Reduction of this bulk will free autoclaves and other decontamination and disposal processes within the laboratory for more rapid and efficient handling of materials known to be contaminated.

Inevitably, disposal of materials raises the question, "How can we be sure that the materials have been treated adequately to assure that their disposal does not constitute a hazard?" In the small laboratory, the problem is often solved by having each investigator disinfect all contaminated materials not of immediate use at the end of each day and place them in suitable containers for routine disposal. In larger laboratories, where the mass of materials for disposal becomes much greater and sterilization bottlenecks occur, materials handling and disposal will likely be the chore of personnel not engaged in the actual research. In either situation, a positive method should be established for designating the state of materials to be disposed. This may consist of a tagging system stating that the materials are either sterile or contaminated.

Disposal of materials from the laboratory and animal holding areas will be required for research projects ranging in size from an individual researcher to those involving large numbers of researchers of many disciplines. Procedures and facilities to accomplish this will range from the simplest to the most elaborate. The primary consideration in any of these is to dispel the notion that laboratory wastes can be disposed of in the same manner, and with as little thought, as household wastes. Selection and enforcement of safe procedures for disposal of laboratory materials are of no less importance than the consideration given to any other methodology for the accomplishment of research objectives.

Materials of dissimilar nature will be common in laboratories studying recombinant DNA molecules. Examples are combination of common flammable solvents, chemical carcinogens, radioactive isotopes, and concentrated viruses or nucleic acids. These may require input from a number of disciplines in arriving at the most practical approach for their decontamination and disposal.

8. Selecting Chemical Disinfectants in Recombinant DNA Research

No single chemical disinfectant or method will be effective or practical for all situations in which decontamination is required. Selection of chemical disinfectants and procedures must be preceded by practical consideration of the purposes for the decontamination and the interacting factors that will ultimately determine how that purpose is to be achieved. Selection of any given procedure will be influenced by the information derived from answers to the following questions:

- What is the target organism(s) (i.e., host, vector, and donor organism from which DNA segments are obtained)?
- What disinfectants, in what form, are known to, or can be expected to, inactivate the target organism(s)?
- What degree of inactivation is required?
- In what menstruum is the organism suspended (i.e., simple or complex, on solid or porous surfaces, and/or airborne)?
- What is the highest concentration of cells anticipated to be encountered?
- Can the disinfectant, either as a liquid, a vapor, or gas, be expected to contact the organisms, and can effective duration of contact be maintained?
- What restrictions apply with respect to compatibility of materials?
- What is the stability of the disinfectant in use concentrations, and does the anticipated use situation require immediate availability of the disinfectant or will sufficient time be available for preparation of the working concentration shortly before its anticipated use?

The primary target of decontamination in the laboratory is the organism(s) under investigation. Laboratory preparations or cultures usually have titers in excess of those normally observed in nature. Inactivation of these materials presents other problems, since agar, proteinaceous nutrients, and cellular materials can effectively retard or chemically bind the active moieties of chemical disinfectants. Such interferences with the desired action of disinfectants may require higher concentrations and longer contact times than those shown to be effective in the test tube. Similarly, a major portion of the contact time required to achieve a given level of agent inactivation may be expended in

inactivating a relatively small number of the more resistant members of the population. The current state of the art provides little information on which to predict the probable virulence of these more resistant cells. These problems are, however, common to all potentially pathogenic agents and must always be considered in selecting disinfectants and procedures for their use.

Organisms exhibit a range of resistance to chemical disinfectants. In terms of practical decontamination, most vegetative bacteria, fungi, and lipid-containing viruses are relatively susceptible to chemical disinfection. The nonlipid-containing viruses and bacteria with a waxy coating, such as tubercle bacillus, occupy a mid-range of resistance. Spore forms are the most resistant.

A disinfectant selected on the basis of its effectiveness against organisms on any range of the resistance scale will be effective against organisms lower on the scale. Therefore, if disinfectants that effectively control spore forms are selected for routine laboratory decontamination, it can be assumed that any other organisms generated by laboratory operations, even in higher concentrations, would also be inactivated.

An additional area that must be considered, and for which there is little published information available, is the "inactivation" of DNA molecules. Strong oxidizers, strong acids and bases, and either gaseous or aqueous formaldehyde, as well as heat sterilization conditions, should react readily with DNA molecules. Chemical disinfectants that are active against the organism from which the DNA is obtained should also be effective in "inactivating" the DNA of the organism. Chemical disinfectants that effectively control spore forms (hypochlorite containing 500 ppm available chlorine and iodophor solution containing 1600 ppm iodine) should be considered excellent candidates for "inactivating" DNA molecules. The ability of disinfectants to destroy the DNA molecule being studied, however, should be confirmed in the experimenter's laboratory.

Pertinent characteristics and potential applications for several categories of chemical disinfectants most likely to be used in the biological laboratory are summarized in the table below. Practical concentrations and contact times that may differ markedly from the recommendations of manufacturers of proprietary products are suggested. It has been assumed that microorganisms will be afforded a high degree of potential protection by organic menstrooms. It has not been assumed that a sterile state will result from application of the indicated concentrations

SUMMARY OF PRACTICAL DISINFECTANTS FOR

DISINFECTANTS		PRACTICAL REQUIREMENTS				INACTIVATES					IMPORTANT CHARACTERISTICS					
		Use Dilution	Contact Time (Minutes)		Temperature, °C	Relative Humidity, %	Vegetative Bacteria	Lipoviruses	Nonlipid Viruses	Bacterial Spores	Effective Shelf Life > 1 week ^c	Corrosive	Flammable	Explosion Potential	Residue	Inactivated by Organic Matter
Lipovirus	Broad Spectrum															
TYPE	CATEGORY															
Liquid	Quat. Ammon. Cpds	0.1 - 2.0%	10	NE			+	+		+					+	+
	Phenolic Cpds	1.0 - 5.0%	10	NE			+	+	b	+				+		
	Chlorine Cpds	500 ppm ^a	10	30			+	+	+	+	+				+	+
	Iodophor	25 - 1600 ppm ^a	10	30			+	+	+	+	+				+	+
	Alcohol, Ethyl	70 - 85%	10	NE			+	+	b		+		+			
	Alcohol, Isopropyl	70 - 85%	10	NE			+	+	b		+		+			
	Formaldehyde	0.2 - 8.0%	10	30			+	+	+	+	+				+	
Glutaraldehyde	2%	10	30			+	+	+	+	+				+		
Gas	Ethylene Oxide	8 - 23 g/ft ³	60	60	37	30	+	+	+	+	NA		+ ^d	+ ^d		+
	Paraformaldehyde	0.3 g/ft ³	60	60	>23	>60	+	+	+	+	NA		+ ^e	+ ^e		+

Note: NA - Not applicable
NE - Not effective

^a Available halogen

^b Variable results dependant on virus

^c Protected from light and air

^d Neither flammable nor explosive in 90% CO₂ or fluorinate hydrocarbon, the usual use form

^e At concentrations of 7 to 73% by volume in air, solid-exposure to open flame

and contact times. It should be emphasized that these data are only indicative of efficacy under artificial test conditions. The efficacy of any of the disinfectants should be conclusively determined by individual investigators. It is readily evident that each of the disinfectants has a range of advantages and disadvantages as well as a range of potential for inactivation of a diverse microflora. Equally evident is the need for compromise as an alternative to maintaining a veritable "drug store" of disinfectants.

USE IN RECOMBINANT DNA RESEARCH

					POTENTIAL APPLICATION										EXAMPLES OF PROPRIETARY DISINFECTANTS ^h	
Compatible For Electronics	Skin Irritant	Eye Irritant	Respiratory Irritant	Toxic ^g	Work Surfaces	Dirty Glassware	Large Area Decon.	Air Handling Systems	Portable Equip. Surface Decon.	Portable Equip. Penetrating Decon.	Fixed Equip. Surface Decon.	Fixed Equip. Penetrating Decon.	Optical & Electronic Instruments	Liquids For Discard	Books, Papers	
+	+	+		+	+	+			+		+					A-33, CDO, End-Bac, Hi-Tor, Mikro-Quat
	+	+		+	+	+			+		+					Hi-Phene, Matar, Mikro-Bac, O-Syl
	+	+	+	+	+	+			+		+			+		Chloramine T, Clorox, Purex
	+	+		+	+	+			+		+					Hy-Sine, Ioprep, Mikroklene, Wescodyne
		+		+	+	+			+		+					
	+	+		+	+	+			+		+					Sterac
	+	+		+	+	+			+		+					Cidex
+	+	+	+	+						+			+		+	Carboxide, Cryoxidide, Steroxidide
+	+	+	+	+			+	+		+		+	+			

^f Usually compatible, but consider interferences from residues and effects on associated materials such as mounting adhesives.

^g By skin or mouth or both - refer to manufacturer's literature and/or Merck Index

^h Space limitations preclude listing all products available. Individual listings (or omissions) do not imply endorsement or rejection of any product by the National Institutes of Health.

9. Caution Required When Applying Disinfectant Methods

a. Heat Sterilization

The hazards of handling hot solids and liquids are reasonably familiar. Laboratory personnel should be cautioned that steam under pressure can be a source of scalding jets if the equipment for its application is mishandled. Loads of manageable size should be used. Fluids treated by steam under pressure may be superheated if removed from the sterilizer too promptly after treatment. This can cause a sudden and violent boiling of the contents from containers that can splash scalding liquids onto personnel handling the containers.

b. Liquid Disinfectants

Particular care should be observed when handling concentrated stock solutions of disinfectants. Personnel assigned the task of making up use-concentrations from stock solutions must be properly informed as to the potential hazards and trained in the safe procedures to follow. The concentrated quaternary and phenolic disinfectants are particularly harmful to the eyes. Even a small droplet splashed in the eyes may cause blindness. Protective face shields and goggles should be used for eye protection, and long-sleeved garments and chemically resistant gloves, aprons, and boots should be worn to protect from corrosive and depigmentation effects to the skin. One of the initial sources for hazard information on any given product will be the label on its container.

c. Vapors and Gases

Avoid inhalation of vapors of formaldehyde and ethylene oxide. Stock containers of these products should be capable of confining these vapors and should be kept in properly ventilated chemical storage areas in the event of inadvertent leakage. In preparing use-dilutions and when applying them, personnel should control the operations to prevent exposure

of others and wear respiratory protection as necessary. Mutagenic potential has been attributed to ethylene oxide; toxic and hypersensitivity effects are well established for formaldehyde.

d. Radiation

The uses of UV irradiation carry the danger of burns to the cornea of the eyes and the skin of persons exposed for even a short time. Proper shielding should be maintained where irradiation treatment is used when personnel and laboratory animals are present. Guard against reflecting surfaces (e.g., polished stainless steel) occurring in line with the light source. In areas irradiated without shielding on special occasions or during off-duty hours, post the area with warning signs to prevent *unscheduled entry of personnel*.

F. Care and Use of Laboratory Animals

Special attention must be given to the humane treatment of all laboratory animals in accordance with the Animal Welfare Act of 1970. The implementing rules and regulations appear in the Code of Federal Regulations (CFR) Title 9, Chapter 1, Subchapter A, Parts 1, 2, and 3. Recommended provisions and practices that meet the requirement of the Act have been published by the U. S. Public Health Service.

Each laboratory should establish procedures to ensure the use of animals that are free of disease prejudicial to the proposed experiments and free from carriers of disease or vectors, such as ectoparasites, which endanger other experimental animals or personnel.

Animal caretakers must be well trained in the basic fundamentals of laboratory animal care. Appropriate training materials are available from a number of animal care associations or commercial organizations.

Animal caretakers, scientists, or others routinely exposed to infected animals, potentially contaminated equipment, and animal wastes should participate in preventive medical and medical surveillance programs of the institution involved.

1. Care and Handling of Infected Animals

Comprehensive reviews indicate that animals infected with a wide range of etiological agents are capable of shedding infectious microorganisms in the saliva, urine, or feces. In the absence of specific information to the contrary, all infected animals should be regarded as potential shedders. Procedures appropriate for the handling of infected animals are given below:

- a. Careful handling procedures should be employed to minimize the dissemination of dust from animal and cage refuse.
- b. Cages should be sterilized by autoclaving. Refuse, bowls, and watering devices should remain in the cage during sterilization.
- c. All watering devices should be of the "non-drip" type.
- d. Cages should be examined each morning and at each feeding time so that dead animals can be removed.
- e. Heavy gloves should be worn when feeding, watering, handling, or removing infected animals. Bare hands should NEVER be placed in the cage to move any object therein.
- f. When animals are to be injected with biohazardous material, the animal caretaker should wear protective gloves and the laboratory workers should wear surgeon's gloves. Animals should be properly restrained (e.g., use of squeeze cage for primate inoculation) or tranquilized to avoid accidents that might result in disseminating biohazardous material, as well as to prevent injury to the animal and to personnel.
- g. Animals exposed to biohazardous aerosols should be housed in ventilated cages, in gastight cabinet systems, or in rooms designed for protection of personnel by use of ventilated suits.

h. Animals inoculated by means other than by aerosols should be housed in equipment suitable for the level of risk involved.

i. Infected animals to be transferred between buildings should be placed in ventilated cages or other aerosol-proof containers.

j. The oversize canine teeth of large monkeys present a particular biting hazard; these are important in the potential transmission of naturally-occurring, and very dangerous, monkey virus infections. Such teeth should be blunted or surgically removed by a veterinarian.

k. Presently available epidemiological data indicate that many zoonotic diseases, including infectious hepatitis and tuberculosis, can be transmitted from nonhuman primates to man. Newly imported animals may be naturally infected with these or other infectious diseases, and persons in close contact with such animals may become infected. The inadvertent transmission of zoonotic diseases from the experimental animal to the animal caretaker should be protected against by the use of personal protective equipment or cage systems designed to contain infectious material at its point of origin. Information concerning the level of hazard associated with work with a wide range of etiological agents and the selection of personal protective equipment and ventilated cage systems can be found in numerous publications.

2. General Guidelines that Apply to Animal Room Maintenance

- a. Doors to animal rooms should be kept closed at all times, except for necessary entrance and exit.
- b. Unauthorized persons should not be permitted to enter animal rooms.
- c. A container of disinfectant, prepared fresh each day, should be kept in each animal room for disinfecting gloves and hands and for general decontamination even though no infectious animals are present. Hands, floors, walls, and cage racks should be washed with an approved disinfectant at the recommended strength as frequently as the supervisor directs.
- d. Floor drains in animal rooms, as well as floor drains throughout the building, should be flooded with water or disinfectant periodically to prevent backup of sewer gases.
- e. Shavings and other refuse on floors should not be washed down the floor drain, because such refuse clogs the sewer lines.
- f. An insect and rodent control program should be maintained in all animal rooms and in animal food storage areas.
- g. Special care should be taken to prevent live animals, especially mice, from finding their way into disposable trash.
- h. Specific instructions involving the housing, care, and maintenance of laboratory animals are available from numerous sources (see Section VI,F, "Reference Bibliography on Biological Safety").

3. Necropsy Rules for Infected Animals

- a. Necropsy of infected animals should be carried out by trained personnel in Biological Safety Cabinets.
- b. Surgeons' gowns should be worn over laboratory clothing during necropsies.
- c. Rubber gloves should be worn when performing necropsies.
- d. The fur of the animal should be wetted with a suitable disinfectant.
- e. Small animals should be pinned down or fastened on wood or metal in a metal tray.
- f. Upon completion of necropsy, all potentially biohazardous material should be placed in suitable containers and sterilized immediately.
- g. Contaminated instruments should be placed in a horizontal bath containing a suitable disinfectant.
- h. The inside of the Biological Safety Cabinets and other potentially contaminated surfaces should be disinfected with a suitable germicide.
- i. Grossly contaminated rubber gloves should be cleaned in disinfectant before removal from the hands, preparatory to sterilization.
- j. Dead animals should be placed in proper leak-proof containers, autoclaved, and properly tagged before being placed outside for removal and incineration.

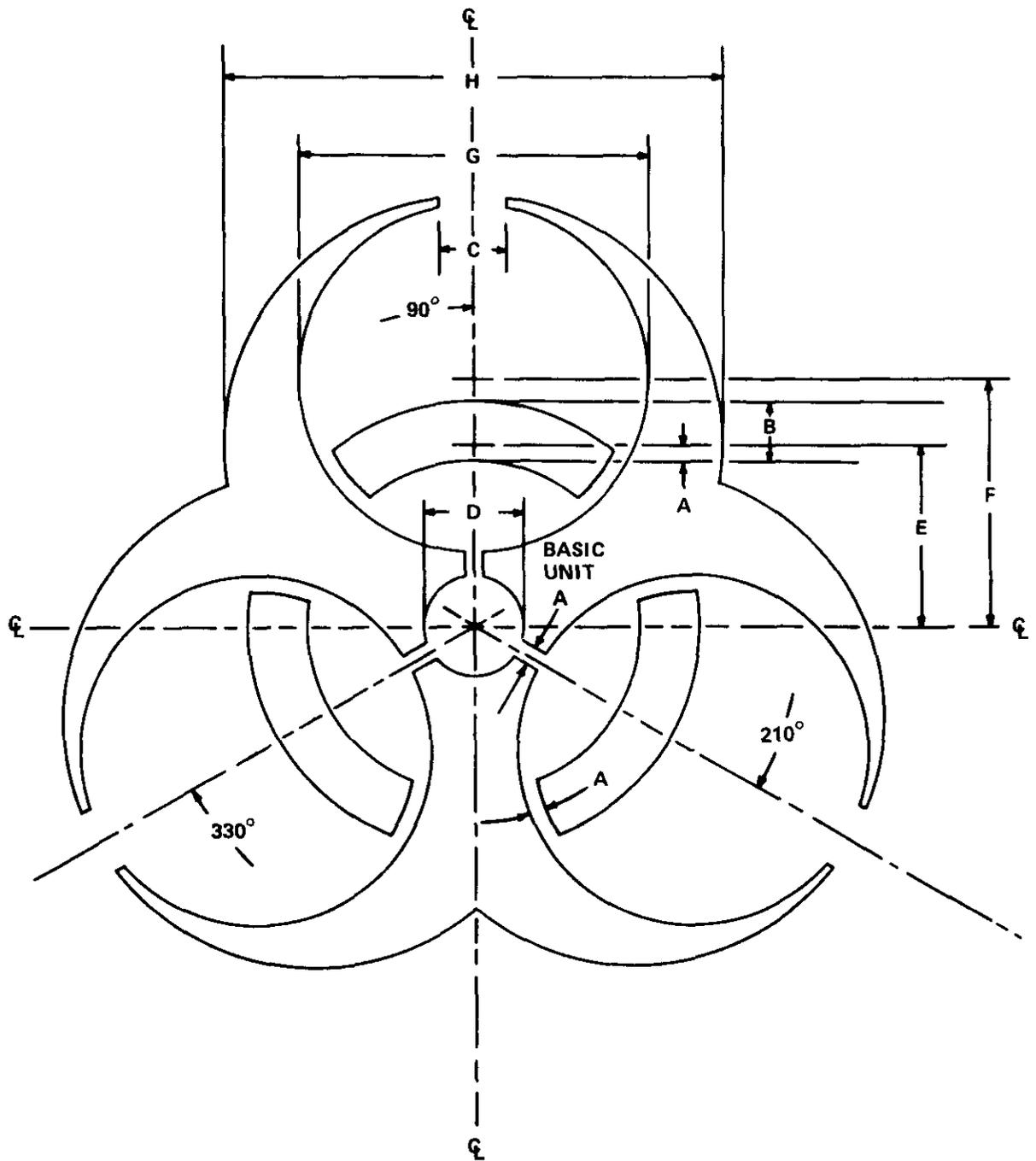
G. Biohazard Symbol and Its Use

A biological hazard symbol is used internationally to indicate the actual or potential presence of a biohazard and to identify equipment, containers, rooms, materials, experimental animals or combinations thereof that contain, or are contaminated with, viable hazardous agents. The symbol can be obtained commercially and placed upon a placard that is large enough for the symbol together with other appropriate information. The term "biohazard" for the purpose of this symbol is defined as "those infectious agents presenting a risk or potential risk to the well-being of man, either directly through his infection or indirectly through disruption of his environment."

The symbol is a fluorescent orange or an orange-red color. There is no requirement for the background color as long as there is sufficient contrast to permit the symbol to be clearly defined. The symbol shall be as prominent as practical, of a size consistent with the size of the equipment or material to which it is affixed, and easily seen from as many directions as possible.

The biohazard symbol is used or displayed only to signify the actual or potential presence of a biological hazard. Appropriate wording may be used in association with the symbol to indicate the nature or identity of the hazard, name of individual responsible for its control, precautionary information, etc., but never should this information be superimposed on the symbol.

Illustrations of the design and proportioning of the symbol and of the symbol used on an access control placard are shown on the following pages. The use of the biohazard symbol in recombinant DNA research is summarized in the table.



DIMENSION	A	B	C	D	E	F	G	H
UNITS	1	3½	4	6	11	15	21	30



BIOHAZARD

ADMITTANCE TO AUTHORIZED PERSONNEL ONLY

Hazard identity: _____

Responsible Investigator: _____

In case of emergency call:

Daytime phone _____ Home phone _____

**Authorization for entrance must be obtained from
the Responsible Investigator named above.**

USE OF BIOHAZARD SYMBOL IN RECOMBINANT DNA RESEARCH

<u>PHYSICAL CONTAINMENT LEVEL</u>	<u>LABORATORY DOOR</u>	<u>ACCESS CONTROL OR CHANGE ROOM DOOR</u>	<u>FREEZERS AND REFRIGERATORS</u>
P1	--	--	--
P2	when experiments are in progress	--	when used to store agents ¹
P3	when experiments are in progress	where agents ¹ are present	when used to store agents ¹
P4	where experiments are conducted	where agents ¹ are present	when used to store agents ¹

¹organisms containing recombinant DNA molecules

H. Protection of Vacuum System When Filtering Biohazardous Materials

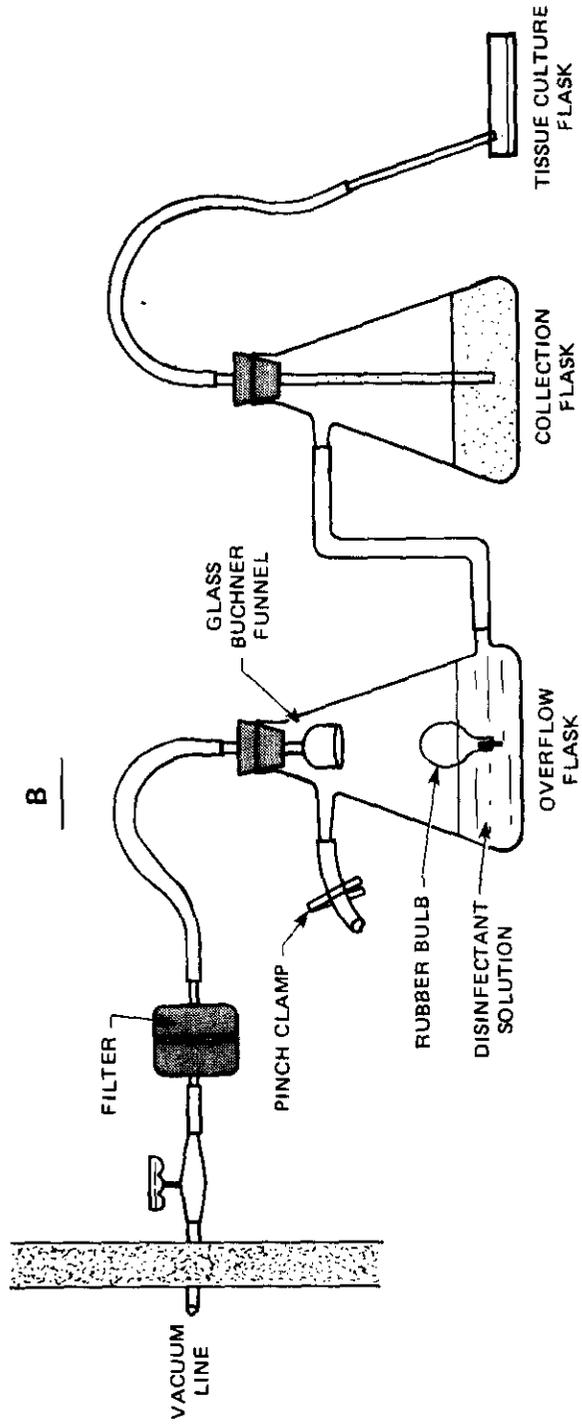
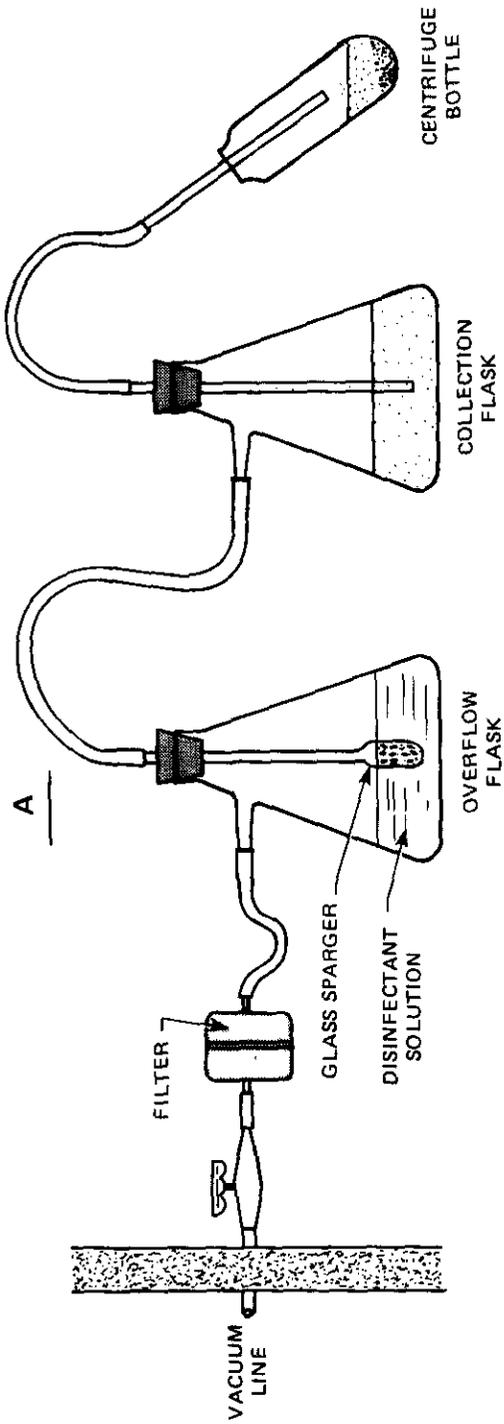
The aspiration of tissue culture media from monolayer cultures and of supernatants from centrifuged samples into primary collection flasks is a common laboratory procedure. Protection should be provided against pulling biohazardous aerosols or overflow fluid into the vacuum system. This protection is provided by the use of an air filter in the line immediately leading into the house vacuum line and an overflow flask for liquids between the collection flask and the air filter.

Two techniques of protecting the vacuum system are shown in the figure. A cartridge-type filter provides an effective barrier to passage of aerosols into the house vacuum system. The filter has a capacity to remove airborne particles 450 nm (0.45 μ) or larger in size. (Ultipor, DFA 3001 AXP5, from the Pall Corporation, Courtland, New York 13045 is an example of such a filter.)

For assembling either apparatus, flexible tubing is used of appropriate inside diameter for the flask and filter fittings and of sufficient wall thickness for the applied vacuum. Filter flasks of capacities from 250 to 4000 ml may be used for the overflow flask, depending on available space and amount of fluid that could be accidentally aspirated out of the collection flask.

The overflow flasks contain a disinfectant solution appropriate for the biohazardous material under study. It is essential that an antifoam, such as Dow Corning Antifoam A, be added to the overflow flask, since bubbling of air through the disinfectant probably will cause considerable foam which, if allowed to reach the filter, will shut off the vacuum.

If the filter becomes contaminated or requires changing, the filter and flask can be safely removed by clamping the line between filter and vacuum source. The filter and flask should be autoclaved before the filter is discarded. A new filter can then be installed and the assembly replaced.



TWO TECHNIQUES FOR PROTECTING VACUUM SYSTEM FROM CONTAMINATION

The apparatus shown in A above is composed of two suction flasks, a filter, rubber stoppers, flexible vacuum tubing, glass tubing, and a small glass sparger. Various small fritted glass or ceramic spargers or gas dispersion tubes are commercially available. The coarse or medium porosity sparger assures that any aerosol passing through the collection flask is dispersed in small bubbles so that adequate contact is made with the disinfectant solutions.

The apparatus depicted in B has the feature of automatically shutting off the vacuum when the storage flask is full. It consists of a 1 L filter flask with a small glass Buchner funnel (15 ml capacity, 29 mm filter disk) inserted upside down in a No. 8 rubber stopper in the mouth of the flask. A hole, 2 cm in diameter, is cut into the bottom of the stopper with a cork borer and of sufficient depth that the filter disc is level with the bottom of the stopper. A 1/2 oz rubber bulb measuring 2 3/8 inches in length and 1 1/4 inches in diameter, with the end plugged with a solid glass rod measuring 1/4 inch in diameter and approximately 2 1/2 inches in length, is placed inside the flask.

If liquids enter the overflow flask, the rubber bulb rises until it presses against the mouth of the Buchner funnel and shuts off the vacuum. The entire unit is autoclavable, but the filter assembly should be thoroughly dried before reuse. A commercial version of this apparatus is available. (Vacuum Guard II, Model VG 201, Spectroderm International, Inc., Fairfax, VA 22030)

III. Containment Equipment

A. The Biological Safety Cabinet

Biological Safety Cabinets are the principal equipment used to provide physical containment. They are used as primary barriers to prevent the escape of aerosols into the laboratory environment. This is an important function, because most laboratory techniques are known to produce inadvertent aerosols that can be readily inhaled by the laboratory worker. Certain cabinets can also protect the experiment from airborne contamination. The selection of a Biological Safety Cabinet is based on the potential hazard of the agent used in the experiment, the potential of the laboratory technique to produce aerosols, and the need to protect the experiment from airborne contamination.

Three types of Biological Safety Cabinets are used in the microbiological laboratory: the Class I cabinet, the Class II cabinet, and the Class III cabinet. The NIH Guidelines for Recombinant DNA Research require that either the Class I or Class II cabinet be used as the primary containment equipment when the P2 or P3 level of physical containment is specified. The Class III cabinet is required at the P4 level of physical containment. The description, capabilities, and limitations of these cabinets follow.

APPLICATIONS OF BIOLOGICAL SAFETY CABINETS IN MICROBIOLOGICAL RESEARCH

Biological Safety Cabinet		Research uses/applications				
Type	Work opening	Face velocity ft/min	Oncogenic viruses ^a	Chemical carcinogens	Etiologic agents ^b	Recombinant DNA
Class I	Glove panel not in place	75	Low and moderate	No	CDC 1-3	P1-P3
	Glove panel in place without gloves	150	Low and moderate	Yes	CDC 1-3	P1-P3
	Glove panel in place with gloves	NA	Low and moderate	Yes	CDC 1-3	P1-P3
Class II Type A	Fixed height usually 10 inches	75, minimum	Low and moderate	No	CDC 1-3	P1-P3
Type B	Sliding sash provides opening adjustable from 8 to 20 inches for introduction and removal of equipment and materials. To obtain proper face velocity, experimentation should be done with 8-inch opening.	100 at 8-inch sash opening	Low and moderate	Yes in low difen and volatility	CDC 1-3	P1-P3
Class III	No direct opening. Access is through double-door sterilizer and decontaminant dunk bath.	NA	Low, moderate and high	Yes	CDC 1-4	P1-P4

^a U.S. Department of Health, Education and Welfare, National Cancer Institute, Office of Research Safety. 1974. Safety standards for research involving oncogenic viruses. Bethesda, Md. 20014. DHEW Publication No. (NIH) 78-790.

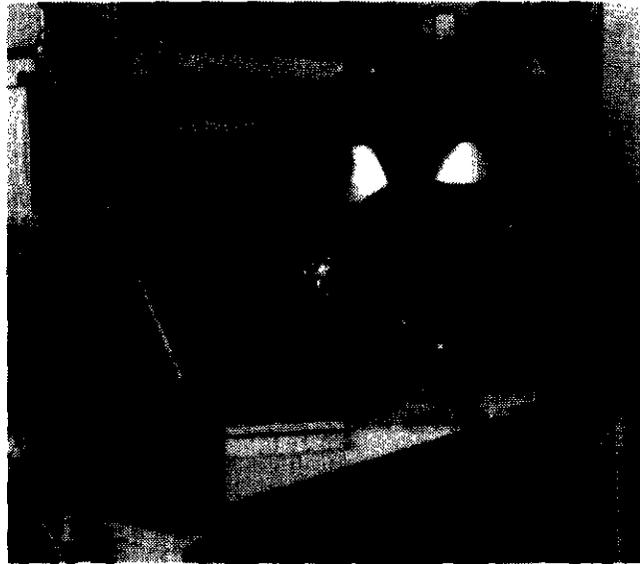
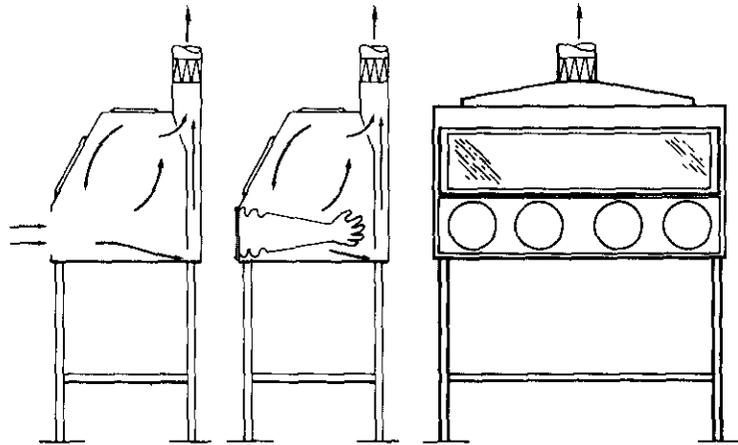
^b U.S. Department of Health, Education and Welfare, Public Health Service, Center for Disease Control. 1976. Classification of etiologic agents on the basis of hazard. Atlanta, Ga. 30333.

1. The Class I Biological Safety Cabinet

The Class I cabinet is a ventilated cabinet that may be used in three operational modes: with a full-width open front, with an installed front closure panel without gloves, and with an installed front closure panel equipped with arm length rubber gloves. Materials may be introduced and removed through the panel opening; and, if provided, through the hinged front view panel or a side UV air lock. Lights, vacuum, gas (do not provide if cabinet is to be operated sealed and with gloves installed), water, and drain can be provided. The materials of construction should be selected to withstand wear, corrosive action of gases and liquids, and decontaminants. Room air flowing into the cabinet prevents the escape of airborne contaminants from the cabinet work area. It flows across the work space, over and under a back wall baffle, out through a HEPA filter and blower in an overhead duct to the building air exhaust system or outdoors. When operated with a full-width open front, a minimum inward face velocity normal to the work opening of at least 75 feet per minute is required.

Protection is provided to the user and the environment, but not to the product (experiment). A wide range of activities is accommodated using equipment as varied as pipetting aids, burettes, pH meters, sonicators, shielded centrifuges, blenders, and lyophilizers. Chemical carcinogens and low levels of radioactive materials and volatile solvents can be used in Class I cabinets with minimum face velocities of 100 ft/min. When these materials are used in the Class I cabinet, a careful evaluation must be made to determine that concentrations do not reach dangerous levels or cause problems of decontamination of the cabinet.

The cabinet is a partial containment unit. Its primary barrier-function can be compromised by the pumping action of sudden withdrawal of the hands, the opening and closing of the room door, or rapid movements past the front of the cabinet. Aerosols created in large quantities, and forcefully, may overcome even higher face velocities. Also, the cabinet does not protect the experimenter's hands and arms from contact with hazardous materials. Such protection is dependent on technique and the use of gloves and other protective clothing.



CLASS I CABINET

2. The Class II Biological Safety Cabinet

The Class II cabinet is commonly known as a laminar airflow Biological Safety Cabinet. Class II cabinets have a front opening for access to the work space and for introduction and removal of materials. Airborne contaminants in the cabinet are prevented from escaping across this opening by a curtain of air formed by (i) unfiltered air flowing from the room into the cabinet and (ii) HEPA filtered air supplied from an overhead grille in the cabinet. This curtain of air also prevents airborne contaminants in the room air from entering the work space of the cabinet across the front opening. The curtain of air is drawn through a grille at the forward edge of the work surface into a plenum below. Air from this plenum is HEPA filtered and recirculated through the overhead grille down into the cabinet. A portion of this filtered air is used to maintain the air curtain and the remainder passes down onto the work surface and is drawn out through grilles at the back edge of the work surface. The HEPA filtered air from the overhead grille flows in a uniform downward movement to minimize air turbulence. It is this air that provides and maintains a clean-air work environment. A percentage of air drawn through the front and back grilles of the work surface, which is equal to the flow of room air into the cabinet, is also filtered by HEPA filters and exhausted from the cabinet.

The selection of utility services and materials of construction are similar to those for Class I cabinets.

There are two types of Class II cabinets, A and B. These differ principally as to:

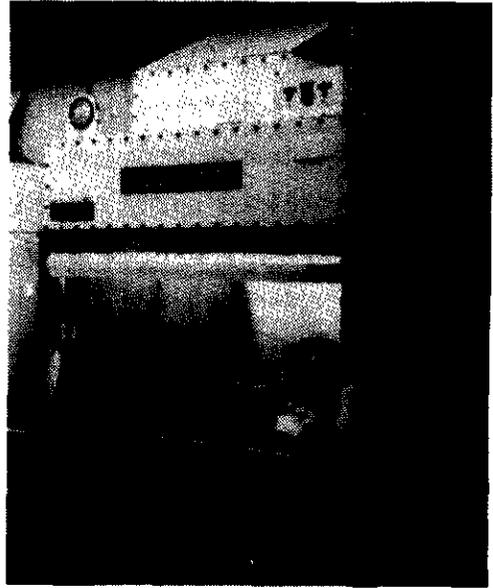
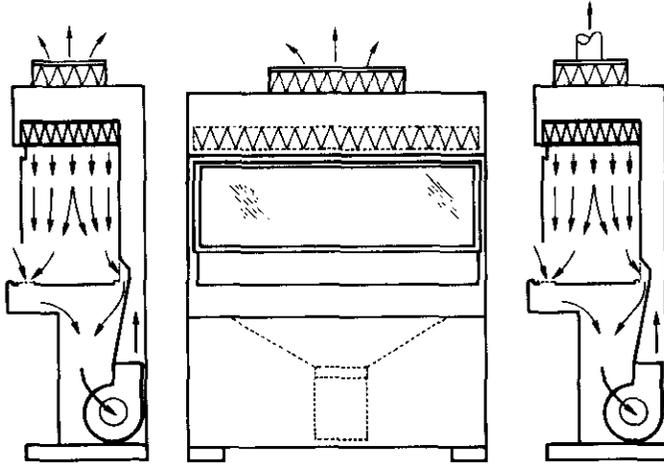
- vertical dimension of the front opening
- proportion of air recirculated
- velocity of airflow to work surface
- manner of discharge of exhaust air
- whether contaminated air plenums are under positive pressure.

The type A cabinet has a fixed front access opening. The inward face velocity through the front opening is at least 75 ft/min. Contaminated air plenums are normally operated at positive pressure. The cabinet

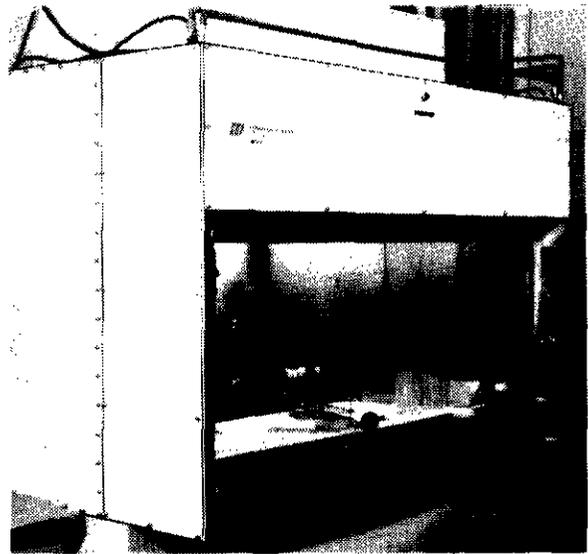
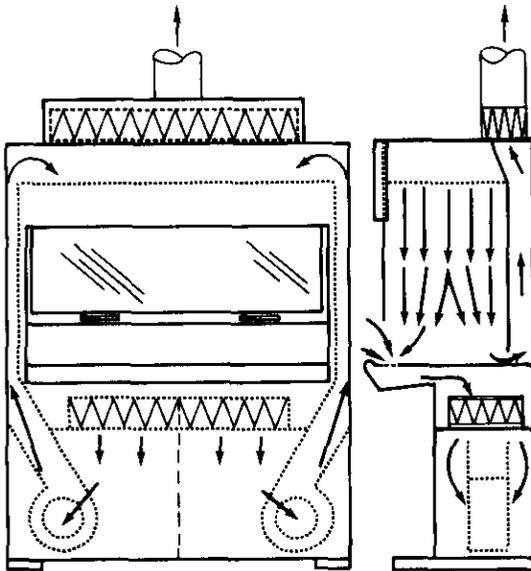
operates with a high percentage (approximately 70%) of recirculated air. The type A cabinets can be operated with recirculation of the filtered exhaust air to the room in which they are located. This minimizes extra demand on supply and exhaust air systems unless the buildup of heat and odor from the recirculated exhaust air requires otherwise.

Type B cabinets do not recirculate their exhaust air to the room. They have a vertical sliding sash rather than the fixed opening of the type A. Inward air velocity of 100 ft/min is attained at an 8-inch sash opening. The cabinet operates with a low percentage (approximately 30%) of recirculated air.

Type A and B cabinets are partial containment units with the same limitations as Class I cabinets. These cabinets provide protection to the user, environment, and product (experiment). Activities are accommodated that use pipetting aids, burettes, pH meters, sonicators, blenders, lyophilizers, and shielded centrifuges. The type B cabinets can be used with dilute preparations of chemical carcinogens, of low-level radioactive materials, and of volatile solvents when the face velocity of 100 ft/min is maintained. When these materials are used, however, a careful evaluation must be made to determine that concentrations do not reach dangerous levels or cause problems of decontamination of the cabinets. The type A cabinets cannot be used with toxic, explosive, flammable, or radioactive substances because of the high percentage of recirculated air.



Type A



Type B

CLASS II CABINETS

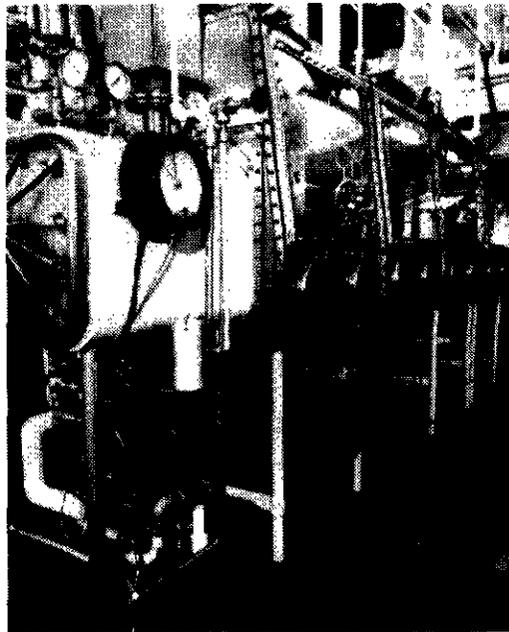
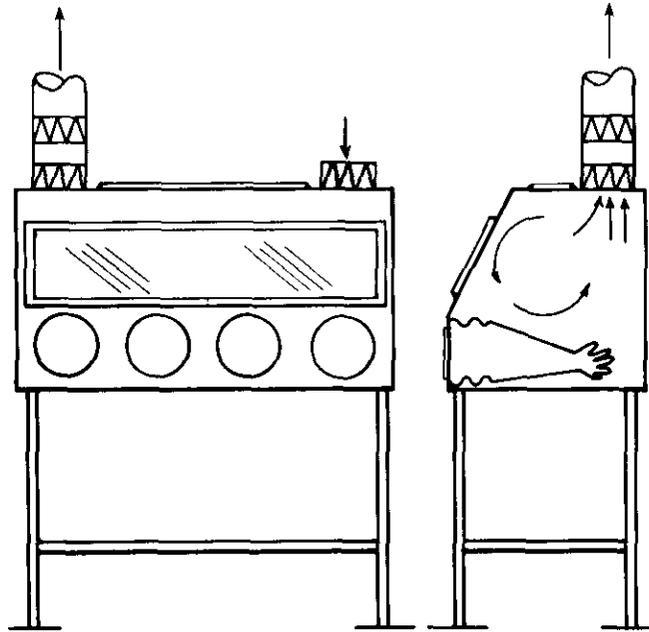
3. The Class III Biological Safety Cabinet

The Class III cabinet is a totally enclosed ventilated cabinet of gastight construction. Operations within the Class III cabinet are conducted through attached rubber gloves. When in use, the Class III cabinet is maintained under negative air pressure of at least 0.5 inches water gage. Supply air is drawn into the cabinet through HEPA filters. The cabinet exhaust air is filtered by two HEPA filters installed in series or one HEPA filter and an incinerator. The exhaust fan for the Class III cabinet is generally separate from the exhaust fans of the facility ventilation system.

Materials are introduced and removed through attached double-door sterilizers and dunk baths with liquid disinfectants. The usual utility services can be provided, but not gas. Liquid wastes go to a holding tank for appropriate decontamination before release into "common" sewage lines. Stainless steel is the usual construction material. Modular designs provide for inclusion of refrigerator, incubator, deep freeze, centrifuge, animal holding, and other special cabinet units.

The Class III cabinet provides the highest level of personnel and environmental protection. Protection is also provided to the product (experiment). Most laboratory activities can be accommodated: the usual cultivation of microorganisms, fertile eggs, tissue cells; microscopy, serology; animal dissections and injections; experimental aerosol exposures; various physical measurements; and many others--on small- to large-scale. Selected gaseous atmospheres can be maintained at desired humidity and temperature conditions.

The Class III cabinet protection can be compromised by puncture of the gloves or accidents creating positive pressure in the cabinet. Flammable solvents should not be used in these cabinets unless a careful evaluation has been made to determine that concentrations do not reach dangerous levels. When required and determined safe, these materials should only be introduced into the system in closed, nonbreakable containers. These materials should not be stored in the cabinet. Electric heaters are preferred over portable, canned-gas heaters. Flammable gas should not be piped to the units.



CLASS III CABINET

B. Certification Procedures

The capability of biological safety cabinets to protect personnel and the environment from exposures to potentially hazardous aerosols is dependent on both the ability of the laboratory worker to use the cabinet properly and the adequate functioning of the cabinet itself. A biological safety cabinet should never be used to contain hazardous materials unless it has been demonstrated to meet certain minimum safety specifications. These specifications are summarized in the table on the next page.

Procedures for certifying the minimum safety specifications of biological safety cabinets are described below. The safety specifications should be certified (i) after a new cabinet has been purchased and installed, but before it is used, (ii) after it has been moved or relocated, and (iii) at least annually.

The certification procedures for Class II cabinets are those recommended by the National Sanitation Foundation in their Standard 49, "Class II (Laminar Flow) Biological Cabinetry." The "NIH Guidelines for Recombinant DNA Research" require that Class II biological safety cabinets conform to all performance requirements specified in Standard 49. Nevertheless, cabinets that have been certified by the National Sanitation Foundation must also be shown to meet the minimum safety specifications once the cabinets have been installed in a laboratory. This demonstration is part of the institutional certification requirement specified by NIH.

MINIMUM PERFORMANCE SPECIFICATIONS OF BIOLOGICAL SAFETY CABINETS

<u>Cabinet</u>	<u>Face velocity, fpm</u>	<u>Velocity profile</u>	<u>Negative pressure, inches, w.g.</u>	<u>Leak tightness</u>	<u>Exhaust filter efficiency</u>
Class I - Open front	75	NA	NA	NA	99.97% for 0.3 μ m particles
Class I - Front panel without gloves	150	NA	NA	NA	"
Class I - Front panel with gloves	NA	NA	$\Delta p > 0.5$	NA	"
Class II - Type A	75	a	NA	Leak rate $< 1 \times 10^{-4}$ cc/sec at 2" w.g. pressure	"
Class II - Type B	100	a	NA	NA	"
Class III	NA	NA	$\Delta p > 0.5$	Leak rate $< 1 \times 10^{-5}$ cc/sec at 3" w.g. pressure	b

a) Dependent on National Sanitation Foundation (NSF) certification in accordance with NSF Standard 49.

b) Both HEPA filters must be certified to have a filtration efficiency of 99.97% for 0.3 μ m particles. When an incinerator is used in lieu of the second HEPA filter, the incinerator must be capable of destroying all spores of *Bacillus subtilis* when challenged at a concentration of 10^5 spores per cubic foot.

1. Certification of the Face Velocity of the Class I Cabinet

a. Equipment Required

A thermoanemometer with a sensitivity of ± 2 lpm or 3 percent of the indicated velocity shall be used.

b. Test Procedure

(1) Take air velocity measurements at the midpoint height approximately one inch behind the vertical plane of the front work access opening.

(2) The individual velocity measurements shall be taken every four inches across the width of the front work access opening but no closer than four inches from edges of the work opening.

c. Test Criterion

The average face velocity through the work access opening shall be at least 75 lpm with no single measurement less than 60 lpm.

2. Certification of the Face Velocity of the Class II, Type A Cabinet

This test is performed to determine the calculated face velocity of the supply air through the work access opening.

a. Equipment Required

A thermoanemometer with a sensitivity of ± 2 lfm or 3 percent of the indicated velocity shall be used.

b. Procedure

(1) The air velocity measurements shall be taken at multiple points across the exhaust filter face on a grid with the points approximately four inches apart and four inches above the face of the filter. The minimum number of air velocity readings shall be nine for each square foot of exhaust filter surface. Using the average air velocity, the exhaust air quantity (cfm) shall be calculated.

(2) Calculate the face velocity of the supply air entering the work access opening by dividing the exhaust airflow quantity by the work access opening area.

c. Test Criterion

The calculated face velocity through the work access opening of the cabinet shall not be less than 75 lfm.

3. Certification of the Face Velocity of the Class II, Type B Cabinet

a. Equipment Required

A thermoanemometer with a sensitivity of ± 2 lfm or 3 percent of the indicated velocity shall be used.

b. Test Procedure

- (1) Turn off fans that recirculate air within the cabinet.
- (2) Close sliding sash to 8-inch opening position.
- (3) Take air velocity measurements at the midpoint height approximately one inch behind the vertical plane of the front work access opening.
- (4) The indicated velocity measurements shall be taken every four inches across the width of the front work access opening but no closer than four inches from edges of the work opening.

c. Test Criterion

The average face velocity through the work access opening shall be at least 100 lfm with no single measurement less than 75 lfm.

4. Certification of the Velocity Profile of the Class II Cabinet

This test is performed to measure the velocity of the air that is recirculated through the overhead grille down into the cabinet.

a. Equipment Required

A thermoanemometer with a sensitivity of ± 2 lpm or 3 percent of the indicated velocity shall be used. Provide stand and clamp to hold the probe.

b. Test Procedure

Measure the air velocity in the work space at multiple points across the work space below the filters on a grid scale to give approximately nine readings per square foot in the horizontal plane defined by the bottom edge of the window frame. Air velocity readings shall be taken at least six inches away from the perimeter walls of the work area. The thermoanemometer probe shall be held by a clamp attached to a stand to eliminate hand movement.

c. Test Criterion

The downward airflow velocity profile through the cross section of the unobstructed work area of the cabinet shall meet the velocity profile as established during the certification process by NSF in accordance with NSF Standard 49. The velocity of any single point shall not be below 45 lpm. For those cabinets manufactured prior to adoption of Standard 49, the average downward airflow velocity shall be 80 lpm with individual point readings not varying more than ± 20 percent of the average.

5. Certification of the Leak Tightness of the Class II, Type A Cabinet

This is to be performed on all contaminated air plenums of Class II cabinets that are under positive pressure with respect to the laboratory room. This test is performed to determine if the exterior joints made by welding, gasketing, penetrations or sealant seams are free of leaks that might release potentially hazardous materials into the room environment.

a. Equipment Required

(1) Industrial type halogen leak detector, General Electric Ferret, G. E. Catalog No. 50-420 810 HFJK or equal.

(2) Calibrated leak standard, G. E. LS-20, Catalog No. 50-420 701 AAAMI ($0-10 \times 10^{-7}$ cc/sec) or equal.

(3) Tank(s) of halide gas (dichlorodifluoromethane).

(4) Manometer, magnehelic gage or U-tube water column (graduated to read in inches water gage).

(5) Gasketed rigid steel plate, four furniture type pipe clamps, four pieces of 4 x 4 x 8' lumber, and assorted tools.

b. Test Procedure

(1) Prepare the test area of the cabinet as a closed system by sealing the front window opening, exhaust port, removable panels, and all other penetrations.

(2) Attach a manometer or pressure gage to the test area to indicate the interior pressure.

(3) Pressurize the test area with air to a reading of two inches water gage. If the test area holds this pressure without loss for 30 minutes, release pressure. If the test area does not hold this pressure, examine for gross leaks with soap solution (1:10 dilution of Ajax liquid dishwashing detergent or equal).

(4) The room in which the testing will be performed shall be free of halogenated compounds, and air movements shall be kept to a minimum. No smoking should take place in the test room.

(5) Pressurize the cabinet test area to two inches water gage pressure using halide gas (dichlorodifluoromethane).

(6) Adjust the halogen leak detector in accordance with the manufacturer's instructions to a sensitivity setting of 4.5×10^{-7} cc/sec.

(7) Move the probe over the seams, joints, utility penetrations, panel gaskets, and other areas of possible leakage. The nozzle of the detector probe shall be held at the surface of the test area so as not to jar the instrument and should be moved over the surface at the rate of about one inch per second, keeping the probe 1/4 to 1/2 inch away from the surface.

c. Test Criterion

Halogen leakage shall not exceed a leak rate of 1×10^{-4} cc/sec at two inches water gage pressure.

The acceptance criterion is based on a halogen leak, which would occur if the cabinet plenum contained 100% halide gas. Since pressurizing the plenum to two inches water gage pressure using halide gas creates a concentration of only 0.5% halide gas, the detector is operated at a sensitivity of 4.5×10^{-7} cc/sec to account for the dilution of the halide gas.

6. Certification of Leak Tightness of the Class III Cabinet Systems

a. Equipment Required

- (1) Industrial type halogen leak detector, General Electric Ferret, G. E. Catalog No. 50-420 810 HFJK or equal.
- (2) Calibrated leak standard, G. E. LS-20, Catalog No. 50-420 701 AAAMI (0-10 x 10⁻⁷ cc/sec) or equal.
- (3) Tank(s) of halide gas (dichlorodifluoromethane).
- (4) Manometer, magnehelic gage or U-tube water column (graduated to read in inches water gage).
- (5) Soap Solution (1:10 dilution of Ajax liquid dishwashing detergent or equal), spray bottles and brushes.
- (6) Glove opening cover plates, silicone rubber sheet gasketing, rigid steel plates, C-type clamps, duct tape, and assorted tools.

b. Test Procedure

- (1) Seal all air inlets and outlets of the cabinet system: Fill with water all deep seal water traps in the cabinet system's waste water drain system. Fill the dunk tank(s) with water. Close all valves of the cabinet system (e.g., waste water drain, vents, air, vacuum, steam, water, etc.). Install gloves or attach and tighten all gasketed glove opening cover plates to the cabinet system. Close and seal the outer sterilizer door(s) located in the system.
- (2) Tape all glass windows with masking tape at 12-inch intervals to prevent possible breakage.
- (3) Provide access means to pressurize the cabinet with air and halide gas. Access may be by hoses passed through the dunk tanks or utility service piping.
- (4) Attach a manometer, magnehelic gage or U-tube water column to the cabinet system in a manner that indicates the differential pressure between the cabinet system and the room.
- (5) Pressurize the cabinet system or section of the system to be tested with air to three-inch water gage and hold the system under

pressure for 30 minutes. If pressure is lost rapidly, check the system for gross leaks. Gross leaks can be found by sound and feel. Repair the gross leaks.

(6) Once the gross leaks have been repaired, smaller leaks can be detected by soap solution testing. Prepare a 1:10 dilution of liquid dishwashing detergent such as Ajax or equal. Pressurize the cabinet system to three inches water gage with air and maintain this pressure. Carefully apply soap solution to all joints, window seals, and penetrations. Repair all leaks indicated by the formation of bubbles. Retest all repaired leaks.

(7) After soap solution testing, thoroughly clean all surfaces to remove trace quantities of the soap solution.

(8) Prepare the Halogen Leak Detector in accordance with the manufacturer's specifications.

(9) Calibrate the leak detector according to the manufacturer's instructions. Use a calibrated halide gas leak standard such as the LS-20. Adjust the leak standard to indicate a leak rate of 1×10^{-7} cc/sec. Using the leak standard, adjust the sensitivity of the instrument to indicate a leak rate of 1×10^{-7} cc/sec on the 0-10 scale. [During the leak testing process, check the sensing instrument against the calibrated leak standard frequently. Excessive exposure of the filament to dichlorodifluoromethane can cause corrosion and desensitization of the filament.]

(10) Before halide gas is added to the system, a complete background scan should be made. The area in which the leak testing is performed must be free of extraneous sources of halogenated compounds, because they will interfere with the sensitivity of the test. Such sources of background could originate from indiscriminate dumping of refrigerant charges, leaky lines, degreasers using halogenated solvents, paint fumes, automobile exhaust, cigarette and pipe smoke, ethylene oxide cylinders and aerosol cans using halogenated gases as the propellant and insulation. Air turbulence should be eliminated to prevent the dilution of any escaping test gas. Background interference may be controlled by maintaining the test

area at a positive air pressure with respect to the surrounding area. This would indicate if the space is free of halogenated compounds or other interfering material. Scan the surfaces of the Class III cabinet system, such as welded seams, gasketed areas, pipe penetrations, valves, windows, control penetrations, electrical fittings and conduits, drive shafts and seals, sterilizer attachment(s), drain lines, vent lines, filter housing, etc. The scanning rate with probe is approximately one inch per second. Mark areas of background interference. The areas of background should be eliminated, if possible.

(11) After the space has been shown to be free of background interference, release into the cabinet system atmosphere, one ounce of the halide gas for each 30 cubic feet of the cabinet system volume. This amount of halide gas will create a concentration of approximately 1% halide gas by volume. After the halide gas has been introduced into the system, bring the total pressure to 3.0" water gage using air. Set the leak detector on the scale that reads 1×10^{-7} cc/sec. (Note: The filament of the leak detector operates at a high temperature and voltage. The following safety precautions must be followed: (i) Never use the leak detector in an environment that contains an explosive vapor. (ii) Never test in vents or enclosed spaces, such as bearing housings, oil tanks or piping, without first testing the area with an explosion meter. (iii) The detector must be grounded.)

(12) Scan all joints, window seals and penetrations. The leak detector probe is held close to the surface to be tested (but not touching) and it should be moved at approximately one inch per second. Mark all points of leakage. Make repairs, retest for background, and then add halide gas and retest. All components of the Class III system, including sterilizers, attached centrifuges, etc., must be tested in this manner.

(13) Continue testing in this manner until the cabinet(s) is leak tight. (Under prolonged testing procedures, gasket material may become saturated with halide gas. Subsequent off-gasing may cause interference.)

c. Test Criterion

Halogen leakage shall not exceed a leak rate of 1×10^{-5} cc/sec at three inches water gage pressure. The acceptance criterion is based on a halogen leak that would occur if the cabinet system contained 100% halide gas. Since the test concentration of halide gas is 1%, the detector is operated at a sensitivity of 1×10^{-7} cc/sec to account for the dilution of the test gas.

7. Leak Testing of High-Efficiency Particulate Air Filters (HEPA)

This test is performed to determine the filtration efficiency of the HEPA filters and the integrity of the filter housings and the filter mounting frames.

a. Equipment Required

(1) Aerosol Photometer with either linear or logarithmic scale. An instrument of this type shall have a threshold sensitivity of at least 10^{-3} micrograms/liter of air for polydispersed liquid aerosol of dioctyl phthalate (DOP) particles and a capacity for measuring an 80-120 micrograms/liter concentration. The DOP polydispersed liquid aerosol has an approximate light-scattering mean droplet-size distribution, as follows:

99+% less than	3.0 μm
50+% " "	0.7 μm
10+% " "	0.4 μm

The instrument shall sample air at a flow rate of one cfm. The aerosol photometer shall be factory calibrated once each calendar year according to the manufacturer's recommended calibration procedures. (Refer to ANSI Standard, N 101.1-1972, Efficiency Testing of Air-Cleaning Systems Containing Devices for Removal of Particles, or its current edition.)

(2) DOP Generator with Laskin Nozzle(s). Liquid DOP is aerosolized by flowing air through the liquid.

(3) Air source to generator. It shall provide a pressure of 20 ± 2 psig and a minimum free airflow through the generator of one cfm/nozzle.

(4) Auxiliary blower, hose and connection fixtures.

(5) Sealant material (RTV type) and closed cell neoprene gasket material.

(6) Various wrenches and hand tools.

b. Test Procedure

(1) Remove the hardware located downstream of the HEPA filter(s). For HEPA filters that cannot be scan tested in their own

housing, remove the filter and install it in a test assembly and follow normal scan testing procedures.

(2) Airflow through the HEPA filters when testing Class II cabinets should be at normal operating velocities. All other HEPA filters will be tested at 20% of rated filter airflow.

(3) Position the DOP generator to introduce air-generated smoke into the area upstream of the filter. Adjust the generator pressure to 20 ± 2 psig with a minimum free airflow through the generator equaling one cfm per nozzle.

(4) Turn on the aerosol photometer and calibrate according to the manufacturer's instructions.

(5) Measure the upstream concentration of DOP.

(a) For linear readout photometers - (graduated 0-100). Use at least one Laskin nozzle per 500 cfm airflow or increment thereof and adjust the instrument to read 100%.

(b) For logarithmic readout photometers - The upstream concentration shall be adjusted, using the instrument calibration curve, to give a concentration of 1×10^4 particles above the minimum sensitivity of the photometer. (REF: Federal Standard 209B, para. 50.1)

(6) Filter Leak Test

(a) Scanning Method

Holding the photometer probe approximately one inch from the filter face on the downstream side, scan the entire surface area and perimeter (filter gasket-frame-housing area) of the filter in slightly overlapping strokes at a traverse rate of not more than ten feet per minute. The photometer sample rate shall equal $1 \pm 10\%$ cfm. When leakage is indicated, repair leaks in the HEPA filter media with silicone RTV sealant. Repair leaks found at the gasket-frame area with laboratory stopcock (silicone) grease. Replace the gasket, if necessary. Retest filter after repair of leaks is completed.

(b) Probe Method

For HEPA filters that cannot be scan tested in-place,

connect auxiliary blower and hose upstream of HEPA filter and introduce air-generated DOP. Insert the photometer probe into the air duct downstream of the HEPA filter installation and measure for total leakage. If the leakage is above the acceptable limit listed in test criteria, retighten filter clamps and retest. If this does not solve the leakage problem, remove the filter and insert the HEPA filter into a test assembly. Scan test the filter face, housing and gasket area. Repair all leaks, and retest. Install filter in its housing and retest.

c. Test Criterion

A HEPA filter and its frame and housing are considered acceptable when no detectable leaks are observed. A detectable leak is defined as either a reading of 0.01% or greater for linear readout photometers or a reading of one scale division or greater for logarithmic readout photometers. Refer to calibration curve for the instrument in use. Generally, 0.01% is one full-scale division above the minimum sensitivity of the logarithmic photometer.

8. Certification of the Operational Negative Air Pressure in the Class III Biological Safety Cabinet System

a. Equipment Required

(1) Magnehelic gages with scale divisions calibrated to read in tenths of an inch water gage installed on Class III cabinets to measure the differential pressure between the cabinet system and the room.

(2) Remote and local alarm sensors to detect a decrease in the cabinet system negative air pressure.

(3) Inclined Manometer (0-1 inch water gage).

b. Test Procedure

(1) Verify that all gages have an accuracy of $\pm 2\%$ for full-scale readings at 70° F. These gages may be tested by comparison with a liquid-filled inclined manometer.

(2) Balance the quantity of air to be exhausted from the Class III cabinet system. This can be determined by measuring the exhaust air quantity in the cabinet system's main exhaust duct. The minimum air change rate within the cabinet system should be ten air changes per hour.

(3) Verify that the magnehelic gages indicate a negative air pressure of at least 0.5 inches water gage once the cabinet system is balanced and the system is operational.

(4) Adjust the remote and local alarm sensors according to the manufacturer's directions to sense an operational negative air pressure drop below 0.25" water gage. The sensors should have a delayed response to allow for a negative air pressure drop when the operator removes his hands from the gloves.

c. Test Criterion

The negative pressure within the cabinet system is acceptable when at least a 0.5 inch water gage negative pressure with respect to the room is maintained at the proper operating air balance.

IV. SPECIAL LABORATORY DESIGN

Recombinant DNA research requiring physical containment at the P1 and P2 levels can be conducted in conventional laboratory facilities that do not require special design considerations. Experiments requiring P3 or P4 physical containment must be conducted in facilities which meet certain minimum design requirements specified in the Guidelines. This section provides further guidance for the design and certification of such facilities.

A. The P3 Facility

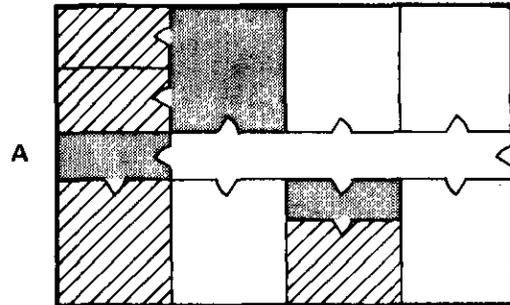
The P3 facility has special engineering features that make it possible for laboratory workers to handle moderately hazardous materials without endangering themselves, other resident personnel, the community, or the environment.

The P3 facility may be a single laboratory module or a complex of modules within a building or an entire building. The P3 facility is separated by a controlled access zone from areas open to the public and other laboratory persons who do not work within the P3 facility. Various arrangements of space can be used to achieve separation as shown in alternatives a, b, and c presented on the following page.

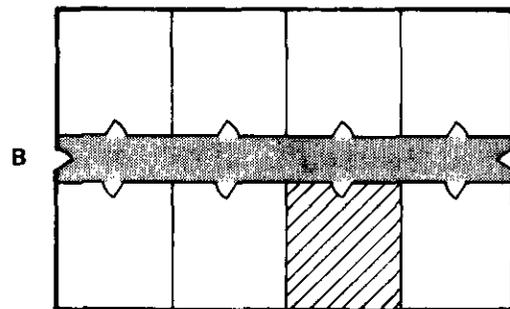
The ventilation system supporting the containment facility is capable of controlling air movement. The direction of airflow is to be from spaces of lower contamination potential to spaces of higher contamination potential. The system is balanced so that there is infiltration of air into each laboratory module or animal room from the adjacent corridors. It is recommended that the infiltration rate be at least 50 cubic feet per minute. The P3 facility may be served by the same supply and exhaust air system that serves areas outside the P3 facility, provided the exhaust air is not recirculated and air balance can be maintained. Air may be recirculated if the air is filtered by HEPA filters. The exhaust air from P3 facilities is discharged to the outdoors clear of occupied buildings and supply air intakes. This is usually accomplished by locating the exhaust stacks on the roof and exhausting upward at relatively high velocity (e.g., >2500 fpm). The general exhaust air can be discharged to the outdoors without filtration or other treatment.

Each laboratory module of the P3 facility should be capable of accommodating a Biological Safety Cabinet. The treated cabinet exhaust air may be discharged directly to the laboratory module. It is recommended, however, that the treated cabinet exhaust air be discharged directly to the outdoors through an individual duct and exhaust fan or through the general exhaust system of the P3 facility. In the latter case, it is important that the exhaust system be designed and operated

Layout A illustrates three approaches to separating a single module P3 facility from a common-use corridor.

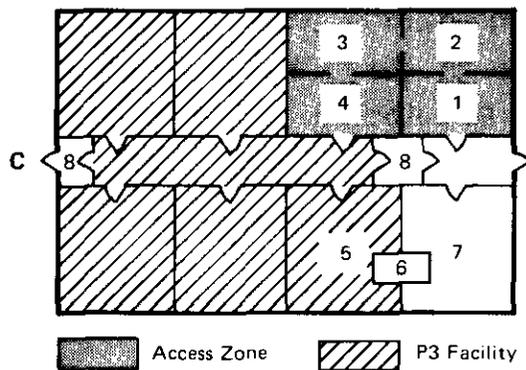


Layout B depicts a corridor as the access zone. This approach is acceptable but undesirable unless strict access control can be ensured.



Layout C shows the access zone as a change room and shower facility. Access to the P3 facility is by passage from the clean clothing change room through the drying room, shower room, and "contaminated" clothing change room. This traverse is reversed for egress. In this example, the airlocks are used only for the passage of equipment, materials, or supplies into the P3 facility.

The change room and shower facility arrangement provides the greatest access control of any of the examples. This arrangement is recommended when the P3 facility comprises a number of laboratory modules or animal rooms.



- 1 Clean Clothing Change Room
- 2 Drying Room
- 3 Shower Room
- 4 Contaminated Clothing Change Room
- 5 Contaminated Waste Handling Room
- 6 Double Door Autoclave
- 7 Washing Room
- 8 Air Lock

**REPRESENTATIVE LAYOUT PLANS FOR
ACCESS CONTROL TO P3 FACILITY**

in a manner that avoids interference with the air balance of the P3 facility and the Biological Safety Cabinet. Pressurization of the exhaust duct must be avoided.

The surface finishes of walls, floors, and ceilings should be resistant to liquid penetration and be readily cleanable. If windows are provided, they should be sealed shut in position. If false ceilings are installed to conceal air ducts and utility distribution lines, they should be constructed of plaster or dry-wall. All ceiling joints should be taped and sealed before the surface finish is applied. The recommended floor surface is a monolithic-type covering that is free of seams or cracks. However, floor tiles with seams sealed by waxing provide an acceptable floor surface.

The openings in walls, floors and ceilings through which utility services and air ducts penetrate should be sealed to permit space decontamination. These openings can be effectively sealed by the application of a liquid silicone plastic.

A foot, elbow, or automatically operated hand washing facility should be provided near the exit area of each primary laboratory module. All doors of the P3 facility should be self-closing.

An autoclave should be located within the P3 facility. With appropriate procedural controls, it is possible to locate the autoclave outside of the P3 facility, provided it is located within the same building.

B. The P4 Facility

The design objective of the P4 facility is to create a facility that will allow the safe conduct of research involving biological agents that may present a high potential hazard to the laboratory worker, or that may cause serious epidemic disease. The distinguishing characteristic of the P4 facility is the provision for secondary barriers that prevent the escape of hazardous materials to the environment. The secondary barriers serve to isolate the laboratory area from the surrounding environment.

The secondary barriers include:

- Monolithic walls, floors, and ceilings in which all penetrations such as air ducts, electrical conduits, and utility pipes, are sealed to ensure the physical isolation of the laboratory area
- Air locks through which supplies and materials can be brought safely into the facility
- Contiguous clothing change rooms and showers through which personnel enter the facility and exit from it
- Double-door autoclaves to sterilize and safely remove wastes and other materials from the facility
- Biowaste treatment system to sterilize liquid wastes
- Separate ventilation system that maintains negative air pressures and directional airflow within the facility
- Treatment system to decontaminate exhaust air before dispersed into the atmosphere.

Although the P4 facility is generally a separate building, it may be constructed as an isolated area within a building. The perimeter wall partitions of the facility should be installed the full height from finished floor to the under surface of the floor or roof above. If windows are installed in the perimeter partitions, they should be fixed shut and the frames should be thoroughly caulked with sealant. The window glass should be safety glass. Perimeter doors should be insect and rodent proof. Wall, floor, and ceiling construction joints, utility pipes and duct penetrations, and electrical conduits and other passages should be sealed to assure isolation of the laboratory environment. The surface finishes should be selected on the basis of their ability to provide a monolithic surface barrier. Epoxy, phenolic, and polyurethane finishes have proved satisfactory for this purpose.

The clothing change rooms and showers are contiguous to the perimeter structure of the facility. They are generally arranged so that the clean clothing change area is separated from the laboratory zone by an air lock or shower area. Personnel egress from the laboratory zone must be through the shower area to the clean clothing change room. Air locks for movement of materials, supplies, and equipment into the facility are also a part of the perimeter structure. The air lock doors should be electrically interlocked so that pressure differentials within the facility can be maintained when the air locks are in use. The double-door autoclave is located so that either the interior or exterior door frame is sealed to the perimeter barrier wall. It is preferable to make the interior door frame contiguous with the barrier wall so that autoclave maintenance can be performed outside the laboratory zone.

The P4 facility is ventilated by its own supply and exhaust air mechanical ventilation system. The system is operated so that the air pressure within the facility can be maintained less than the air pressure outside the perimeter walls. The air system is balanced so that airflow within the facility is from areas with the least hazard potential to areas with the greatest hazard potential.

The air-handling system should provide an air supply consisting of 100 percent outdoor air on a year-round basis. The system should provide separate branch supply and exhaust air ducts to each space to permit proper air balance. The air ducting should be tightly constructed to ensure control of air balance. The supply and exhaust fans should be interlocked to prevent pressurization in the event of exhaust fan failure.

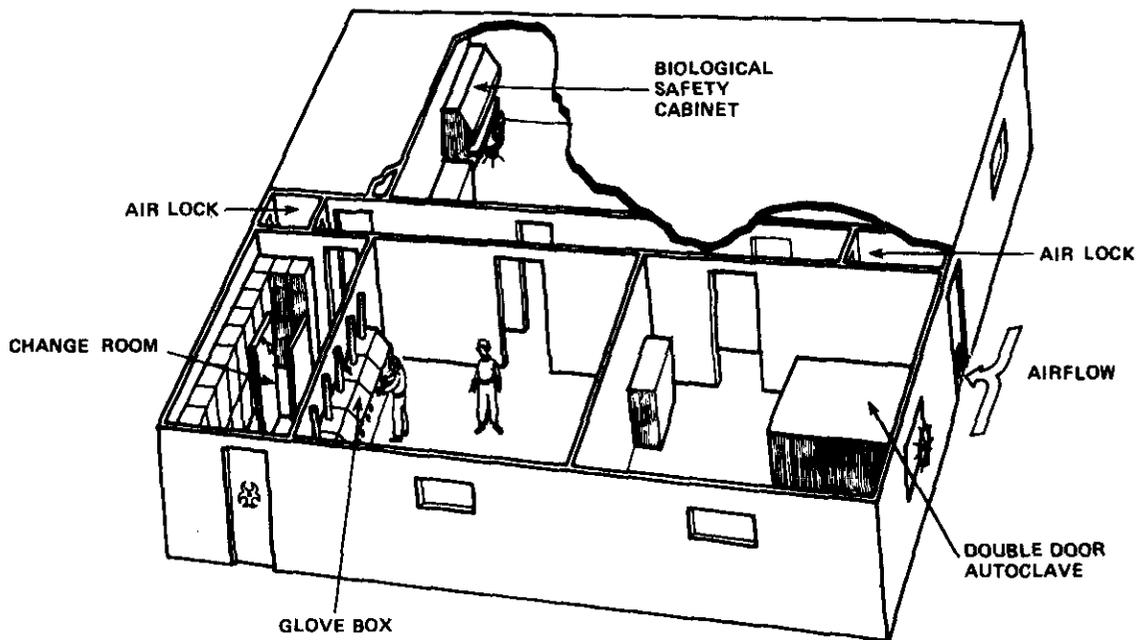
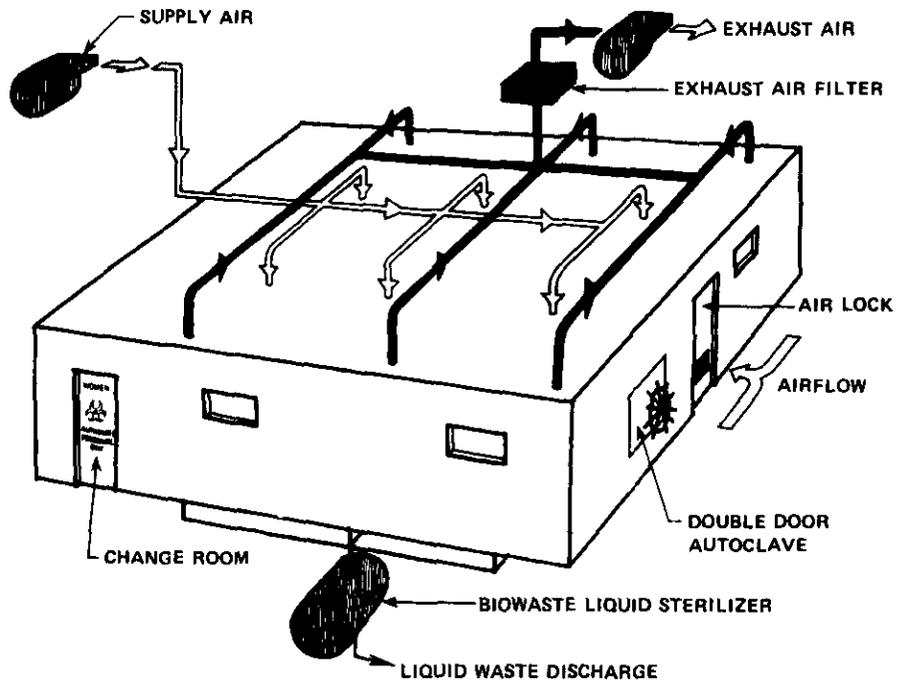
The general exhaust air is filtered by passage through high-efficiency particulate air (HEPA) filters before being discharged to the outdoors. The air filters should be located as near to the laboratory module as possible to minimize the length of potentially contaminated air ducts. The filter plenums should be designed to facilitate (i) testing of filters after installation, and (ii) in-place decontamination before filter removal and replacement.

Mechanical systems should be designed so that maintenance of building machinery, piping, and controls can be performed from outside the laboratory environment.

Liquid effluents from the P4 facility should be collected and decontaminated before disposal into the sanitary sewers. Effluents from laboratory sinks, cabinets, floors, and autoclaves should be sterilized by heat treatment. Liquid wastes from the shower room may be decontaminated with chemical disinfectants (see Section II,E). The wastes from toilets may be discharged directly into the sanitary sewers.

The figure on the following page shows the secondary barriers of the P4 facility.

Primary protection for the laboratory worker within the P4 facility is provided by the use of Class III Biological Safety Cabinets. The exhaust fans for the Class III cabinets are separate from the exhaust fans of the facility ventilation system.



SECONDARY BARRIERS IN REPRESENTATIVE P4 FACILITY

Primary protection may also be provided by having the laboratory worker wear a one-piece positive pressure suit while working in a specially designed suit area within the P4 facility. The suit area is isolated from other areas of the P4 facility by an air lock fitted with airtight doors, a double-door autoclave, and a chemical disinfectant shower. The air pressure within the suit area is less than that in any adjacent area. The exhaust air from the suit area is separately filtered through two sets of HEPA filters installed in series, or filtered by a single HEPA filter, then incinerated before being discharged to the atmosphere. A duplicate filtration system and exhaust fan is provided. An emergency power source to operate the exhaust fans is also provided. The interior surfaces of the suit area have monolithic finishes, and all penetrations for utility services and air ducts through walls, floors and ceilings are sealed.

C. Certification Procedures

Safe conduct of recombinant DNA research is dependent, in part, on the design and operation of the research facility. Facilities that support research at the P3 and P4 physical containment levels must provide certain facility "barrier" systems or safeguards that serve to protect persons and the environment outside of the laboratory setting from potential hazards associated with research. The appropriateness of a facility to support recombinant DNA research is, therefore, dependent on the performance of these facility safeguards.

This section describes the minimum certification requirements for P3 and P4 facilities. These requirements are summarized in the following table. It is also important that all mechanical systems and equipment of the facility are operating satisfactorily and that appropriate maintenance is provided to insure continuous satisfactory operation.

Adaptation or development of new procedures for certification are encouraged for situations where these procedures may not be applicable or best suited. A modified or new procedure would be acceptable provided it is capable of demonstrating that the criteria for certification are achieved.

MINIMUM CERTIFICATION REQUIREMENTS FOR P3 AND P4 FACILITIES

Facility Barrier System	P3 Facility	P4 Facility	P4 suit room with Primary Barriers	P4 suit room without Primary Barriers
Access control	Comply conceptually with any arrangement in the figures on page 149	Contiguous change room/shower facility	Airtight air lock with chemical shower	Airtight air lock with chemical shower
Penetration seals	Integrity demonstrated by visual onsite inspection	Integrity demonstrated by visual onsite inspection	Integrity demonstrated by visual onsite inspection	Integrity demonstrated by field tests (see certification procedure 1)
Directional airflow	Performance demonstrated by field tests (see certification procedure 2)	Performance demonstrated by field tests (see certification procedure 2)	—	—
Negative air pressure	—	—	Negative with respect to all adjacent areas as demonstrated by field measurements (see certification procedure 3)	Negative with respect to all adjacent areas as demonstrated by field measurements (see certification procedure 3)
Exhaust air ducts (layout)	No cross connection with supply ducts as demonstrated by on-site inspection	No cross connection with supply ducts or exhaust ducts from non-P4 areas as demonstrated by on-site inspection	Isolated exhaust system as demonstrated by onsite inspection	Isolated exhaust system as demonstrated by onsite inspection
Exhaust air ducts (tightness)				
existing installation	—	Sufficiently tight construction to assure directional airflow	Sufficiently tight construction to assure negative air pressure	Integrity demonstrated by field tests (see certification procedure 4)
new construction	—	Integrity demonstrated by field tests (see certification procedure 5)	Integrity demonstrated by field tests (see certification procedure 5)	Integrity demonstrated by field tests (see certification procedure 4)
Steam and Ethylene Oxide Sterilizers	Performance demonstrated by field tests (see certification procedure 6)	Performance demonstrated by field tests (see certification procedure 6)	Performance demonstrated by field tests (see certification procedure 6)	Performance demonstrated by field tests (see certification procedure 6)
Exhaust air filter efficiency	—	Performance demonstrated by field tests (see certification procedure 7)	Performance demonstrated by field tests (see certification procedure 7)	Performance demonstrated by field tests (see certification procedure 7)
Biowaste treatment facility	—	Performance demonstrated by field tests (see certification procedure 8)	—	—

1. Certification of Leak Tightness of Penetration Seals For P4 Suit Rooms Without Primary Barriers

This test should be performed to verify the tightness of penetration seals of a P4 suit area when this area is to be used to contain hazardous microorganisms outside of Biological Safety Cabinets or other primary barriers. The purpose of this test is to demonstrate the integrity of all seals for penetrations of pipes, ducts, electrical conduits, etc., where they penetrate walls, floors and ceilings of the P4 suit area. (For P3 and P4 facilities, and P4 suit areas in which potentially hazardous agents are to be confined in primary barriers, it is sufficient to judge the integrity of penetration seals by visual inspection. Acceptance, in this case, would be based on the absence of visual openings around pipes, ducts, conduits, etc., where they pass through walls, floors and ceilings of the facility.)

a. Equipment Required

- (1) Industrial type halogen leak detector, General Electric Ferret, G. E. Catalog No. 50-420-810 HFJK or equal.
- (2) Calibrated leak standard, General Electric LS-20, Catalog No. 50-420 701AAAMI ($0-10 \times 10^{-7}$ cc/sec) or equal.
- (3) Tank(s) of halide gas (dichlorodifluoromethane).
- (4) Respirator equipped with a cartridge for organic vapor removal to be worn by person generating gas on high pressure side.
- (5) Two-way communication system.

b. Test Procedure

- (1) Remove halogenated compounds from the test area.
- (2) Calibrate the leak detector according to the manufacturer's instructions. Adjust the leak standard to indicate a leak rate of 1×10^{-4} cc/sec.
- (3) Prior to testing, perform a background scan of the area to insure the atmosphere is free of halogenated compounds.

(4) Verify that the air pressure in the laboratory area to be tested is negative with respect to all adjacent areas.

(5) Form a "tent" using plastic sheeting around the penetration area on the high pressure side of the wall, floor or ceiling.

(6) Introduce into the "tent" sufficient halide gas (dichlorodifluoromethane) to produce a "cloud" of gas around the penetration.

(7) On the low pressure side of the penetration area, scan the entire seal with the probe of the halogen leak detector.

c. Test Criterion

No halogen leakage shall be detected when the halogen leak detector is set at a sensitivity of 1×10^{-4} cc/sec.

2. Certification of Directional Airflow

This test is conducted to verify that the movement of air is from spaces of lower contamination potential to spaces of higher contamination potential. For example, air should always move from access corridors into laboratory modules.

a. Equipment Required

(1) Mine Safety Appliance Company, Inc., ventilation smoke tubes #5645 or equal.

(2) Space plans of test area.

b. Procedure

(1) Indicate on floor space plans of facility test area the required direction of airflow across each door of the test area.

(2) Verify that the air-handling system supporting the facility is operating normally.

(3) Close all doors of the facility test area.

(4) Determine the direction of airflow across a doorway by opening the door about one inch and holding the smoke tube vertically in the door opening. Observe the direction of smoke movement. Test one door at a time.

(5) Verify that the actual direction of airflow is in accordance with the required direction of airflow as indicated on the space plans. Where this condition is not met, the air-handling system should be rebalanced and the test repeated until the required direction of airflow is achieved.

c. Test Criterion

Movement of air is from spaces of lower contamination potential to spaces of higher contamination potential.

3. Certification of Negative Pressure Within P4 Suit Areas

This test is to verify that the air pressure within the P4 suit area is less than that in all spaces immediately adjacent to the suit area. Magnehelic gages are to be permanently installed so that the pressure differential between all adjacent spaces and the suit area can be continuously monitored.

a. Equipment Required

(1) Magnehelic gages with scale divisions calibrated to read in hundredths of an inch water gage. An appropriate number of gages are to be installed so that the pressure differential between all adjacent spaces and the suit room can be measured.

(2) Inclined manometer (0-0.5 inch water gage).

b. Test Procedure

(1) Verify that all gages have an accuracy of $\pm 2\%$ for full-scale readings at 70°F. These gages may be tested by comparison with a liquid-filled inclined manometer.

(2) Balance the air-handling system of the P4 suit area and the P4 facility.

(3) Measure the air pressure differential as indicated on each magnehelic gage.

c. Test Criterion

The negative pressure within the P4 suit area is acceptable when it is below that of all spaces immediately adjacent to the suit area.

4. Certification of the Leak Tightness of Exhaust Air Ducts from the P4 Suit Area

The purpose of this test is to demonstrate that the exhaust air ducts from P4 suit areas in which work is conducted on the open bench are leak tight. This test is to be conducted for the general exhaust ducts that run from the suit area to and including the "clean" side of the HEPA filter plenums.

a. Equipment Required

- (1) Industrial-type halogen leak detector, General Electric Ferret, G. E. Catalog No. 50-420-810 HFJK or equal.
- (2) Calibrated leak standard, General Electric LS-20, Catalog No. 50-420 701 AAAMI ($0-10 \times 10^{-7}$ cc/sec) or equal.
- (3) Tank(s) of halide gas (dichlorodifluoromethane).
- (4) Manometer, magnehelic gage or U-tube water column (graduated to read in inches water gage).
- (5) Plates to close off and seal all openings in the duct section to be tested.
- (6) A source of air pressure (i.e., portable or tank type vacuum cleaner; high pressure blower).

b. Test Procedure

- (1) Shut down exhaust fan and close off and seal openings in the duct section to be tested.
- (2) Attach a manometer or pressure gage to the duct section to be tested.
- (3) Provide access means to introduce halide gas to pressurize the duct with air.
- (4) Remove all halogenated compounds from the vicinity of the test area.
- (5) Calibrate the leak detector according to the manufacturer's instructions. Adjust the leak standard to indicate a leak rate of 1×10^{-4} cc/sec.

(6) Prior to testing, perform a background scan of the area to insure the atmosphere is free of halogenated compounds.

(7) After the space has been shown to be free of background interference, release into the duct section to be tested, one ounce of the halide gas for each 30 cubic feet of duct volume. This amount of halide gas will create a concentration of approximately 1% halide gas by volume. After the halide gas has been introduced into the duct, bring the total pressure to three inches water gage using air.

(8) Scan all joints, seams, flanges, etc., of the duct. The leak detector probe is held close to the surface to be tested (but not touching) and it should be moved at approximately one inch per second. Mark all points of leakage. Make repairs, retest for background, and then add halide gas and retest.

(9) Continue testing in this manner until the entire duct is leak tight.

c. Test Criterion

No halogen leakage shall be detected when the halogen leak detector is set at a sensitivity of 1×10^{-4} cc/sec.

5. Certification of Leak Tightness of General Exhaust Air Ducts
(New Construction)

The purpose of this test is to demonstrate that newly constructed general exhaust air ducts of P4 facilities are sufficiently airtight. This is important to insure the effective control of air balance and to reduce the potential for escape of airborne contaminants (across duct seams, joints, flanges, etc.) in the event of fan failure. This test is to be conducted for the general exhaust air ducts that run from the individual areas of the P4 facility to and including the "clean" side of the HEPA filter plenum. This test does not apply to exhaust ducts from Class III cabinets. Contaminated exhaust ducts from Class III cabinets to the clean side of the second HEPA filter or incinerator shall meet the same leak tightness requirements specified for Class III cabinets (Section III, B, 6). The test given here was adopted from the "High Pressure Duct Construction Standards," Sheet Metal and Air Conditioning Contractors National Association, Inc., Third Edition, 1975.

a. Equipment Required

(1) A source of air pressure (i.e., portable or tank type vacuum cleaner; high pressure blowers).

(2) A flow-measuring device, usually an orifice assembly consisting of straightening vanes and an orifice plate mounted in a straight tube with properly located pressure taps. Each orifice assembly is accurately calibrated with its own calibration curve. Pressure and flow readings are usually taken with U-tube manometers.

(3) A typical test apparatus is shown in the figure that follows the next page.

b. Procedure

(1) With the air-handling system operating normally, determine the negative pressure at the "contaminated" side of the HEPA filter plenum.

(2) Shut down fan and close off and seal all openings in the duct section to be tested. Connect the test apparatus to the duct by means of a section of flexible duct.

(3) Start the air source with its control damper closed.

(4) Gradually open the inlet damper until the duct pressure reaches a positive pressure equivalent to 2 inches water gage plus the numerical value of the negative pressure as determined in step (1) (e.g., if the negative pressure measured at the "contaminated" side of the HEPA filter plenum were 4 inches water gage, then the test positive pressure should be 6 inches water gage). The test pressure is read on Manometer No. 1 shown in the following illustration. Note that the pressure is indicated by the difference in level between the two legs of the manometer and not by the distance from zero to the reading on one leg only.

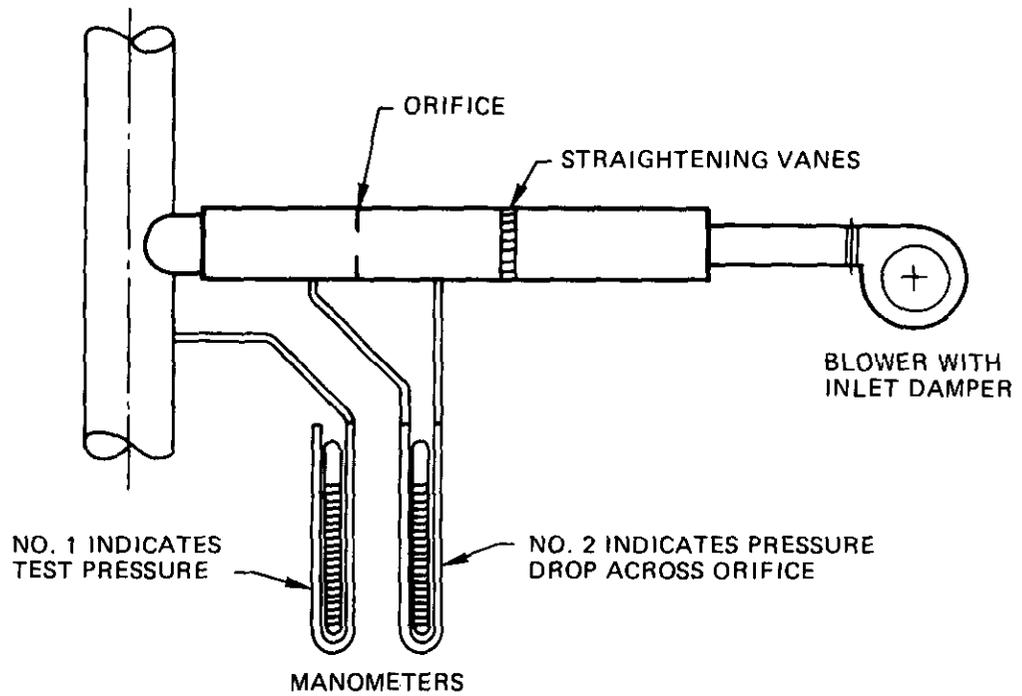
(5) Survey all joints listening for audible leaks. Mark each leak and repair after shutting down test blower.

(6) After all audible leaks have been repaired, reestablish test pressure.

(7) Read the pressure differential across the orifice on Manometer No. 2. The leakage rate in cfm is read directly from the calibration curve for the test orifice plate.

c. Test Criterion

Total allowable leakage under conditions of the test should not exceed one percent of the total system design airflow rate. When partial sections of the duct system are tested, the summation of the leakage for all sections shall not exceed the total allowable leakage.



MANOMETERS USED FOR LEAK TIGHTNESS TESTING

Courtesy of SMACNA High Pressure
Duct Standards - 3rd. Ed.

6. Certification of Steam and Ethylene Oxide Sterilizers

Steam sterilizers are important barrier systems used in research with potentially hazardous microorganisms. They are used as the principal devices for sterilizing contaminated wastes to insure safe disposal. Ethylene oxide sterilizers may also be used in certain applications where items to be sterilized may be adversely affected by steam sterilization conditions. Good safety management requires that the efficacy of these sterilization devices be verified before they are used for the sterilization of materials contaminated with potentially hazardous microorganisms. The tests described here are designed to demonstrate the performance of steam and ethylene oxide sterilizers.

a. Equipment and Materials Required

(1) Spore strips containing both Bacillus subtilis var. niger and Bacillus stearothermophilus. (Amsco's Spordi^R or equal)

Employ separate spore strips with an average certified population of 10,000 B. stearothermophilus and 1,000,000 B. subtilis spores, adjusted to the following resistance data.

<u>Test Organism</u>	<u>Sterilization Medium</u>	<u>Exposure Time & Temperature (°F)</u>	
		<u>Survives</u>	<u>Killed</u>
<u>B. stearothermophilus</u>	Steam	250°, 5 min.	250°, 13 min.
<u>B. subtilis</u>	EtO	15 min.	1 hour, 45 min.

(2) Temperature indicator with remote probes.

(3) Hand towels, 16 x 24 inches.

(4) Stainless steel pan approximately 12" x 18" x 2" deep.

(5) Supporting laboratory equipment (incubator, refrigerator, culture media, miscellaneous glassware).

b. Steam Sterilizer Test Procedure

(1) Fold in half three hand towels and stack them in the stainless steel pan. Place one test spore strip into the fold of the top and bottom towels. Do not remove the spore strips from their glassine envelopes.

(2) Place the temperature probe into the folds of the center towel with the lead extended over the lip of the pan. Place a second probe in the sterilizer drain. Position the pan in the rear center of the sterilizer away from the steam inlet. Pass the temperature leads out of the sterilizer chamber and connect to the recorder.

(3) Close the door, taking care not to cut the probe lead wires.

(4) Operate the sterilizer in accordance with the manufacturer's instructions. The cycle (time and temperature of exposure) shall be set as follows:

Set the minimum time that is required to kill the test spore strips located in the test pan. Use approximately the "kill" time and temperature established above once the temperature indicator located in the test pan reaches 250°F or 121°C.

(5) Record the temperature readings from the indicator (probe leads inside the sterilizer) at three-minute intervals. Simultaneously, record the chamber temperature, chamber pressure, and jacket pressure as shown by the sterilizer indicator.

(6) Upon completion of the cycle, rapidly exhaust the chamber and then remove the test spore strips from the sterilizer.

(7) Aseptically remove all test spore strips and two unheated control strips from their glassine envelopes with sterile forceps and place in previously prepared 12 x 150 mm tubes containing 10 ml of sterile Trypticase Soy Broth.

(8) Incubate one set of test and control tubes for seven days at 55°C for Bacillus stearothermophilus detection. Incubate the second set of test and control tubes for seven days at 37°C for Bacillus subtilis var. niger detection.

(9) All test organisms on each test strip must be killed (i.e., no growth may be visually present after incubation). The control strip must show positive results after incubation.

(10) In the event of test failure, corrective action (e.g., readjustment of steam sterilizer time/temperature) must be undertaken. The test must then be repeated to ensure that the adjustment was successful.

c. Ethylene Oxide Sterilizer Test Procedure

(1) Fold in half three hand towels and stack them in the stainless steel pan. Place one test spore strip into the fold of the top and bottom towels. Do not remove the spore strips from their glassine envelopes.

(2) Position the pan in the rear center of the sterilizer away from the gas inlet.

(3) Operate the sterilizer in accordance with the manufacturer's instructions.

(4) Upon completion of the gas cycle, rapidly exhaust the chamber and then remove the test spore strips from the sterilizer.

(5) Aseptically remove all test spore strips and two unexposed control strips from their glassine envelopes with sterile forceps and place in previously prepared 12 x 150 mm tubes containing 10 ml of sterile Trypticase Soy Broth.

(6) Incubate one set of test and control tubes for seven days at 55°C for Bacillus stearothermophilus detection. Incubate the second set of test and control tubes for seven days at 37°C for Bacillus subtilis var. niger detection.

(7) All test organisms on each test strip must be killed (i.e., no growth may be visually present after incubation). The control strip must show positive results after incubation.

(8) In the event of test failure, corrective action (readjustment of gas concentration and/or exposure time) must be undertaken. The test must then be repeated to ensure that the adjustment was successful.

d. Test Criterion

All spores on each test strip must be killed.

7. Leak Testing of High-Efficiency Particulate Air (HEPA) Filters

The general exhaust air from P4 facilities is filtered by passage through high-efficiency particulate air (HEPA) filters before being discharged to the outdoors. The capability of these filters and their housings and mounting frames to prevent the escape of potential airborne contaminants must be demonstrated as part of the certification for P4 facilities. The acceptance criterion is that there be no detectable leaks when tested by the method described in Section III,B,7, entitled "Leak Testing of High-Efficiency Particulate Air Filters (HEPA)."

- a. Equipment Required - (Section III,B,7,a, page 142)
- b. Test Procedure - (Section III,B,7,b, page 142)
- c. Test Criterion - (Section III,B,7,c, page 144)

8. Certification of the P4 Facility Liquid Biowaste Treatment System

Two types of biowaste treatment systems are available to sterilize liquid effluent from the P4 facility. One system is designed and operated as a continuous flow heat exchange sterilization system. This system consists of coiled tubing that passes through a sealed heat exchange shell. Liquid effluent storage tanks are required to collect untreated liquid effluent and to insure continuous operation of the system over a fixed period of time. To conserve energy, an efficient heat exchange unit is necessary. This system is recommended when the effluent flow rate is in excess of 25 gpm. The second system is a pressure-rated effluent batch sterilization tank. The effluent in this type tank may be heated by injecting steam into a steam jacket or an internal steam coil, by immersed electrical resistance coils, or by oil- or gas-operated burners. Two full-sized batch tanks are required so that while one tank is being used to sterilize the effluent from the P4 facility the other is being used to collect effluent from the facility. Both systems must be designed and equipped with a sealed sampling system to facilitate certification testing.

This certification test is to be conducted to verify the sterilization efficacy of the P4 Biowaste Treatment System. Once this has been proven for the system, the operating temperature, pressure, and quantity or flow rates used for the successful test are to be established as the standard operating conditions for the system.

a. Equipment and Materials Required

- (1) Stock concentrations of Bacillus subtilis var. niger spores.
- (2) Thermocouples and gages for insertion into the biowaste system to record temperature, pressure, quantity, and/or flow rates.
- (3) Equipment to take periodic samples from the sampling system (sterile needles and syringes, decontaminating chemical solutions, sterile transfer containers).
- (4) Other support laboratory equipment (culture media, pipettes, accessibility to an autoclave, incubator, etc.).

b. Test Procedure

(1) Determine and prepare the quantity and concentration of B. subtilis spore suspension required to test the biowaste treatment system. The final test concentration of the challenge liquid waste is to be approximately 1×10^5 spores/ml.

(2) Sterilize or chemically disinfect the sampling system and verify that the system is free of viable contamination.

(3) Fill the biowaste treatment system to operating capacity with water.

(4) Add the spore suspension to the system in a manner to effect adequate mixing of the bacterial spores in the challenge liquid waste.

(5) Take control samples of both the initial stock spore suspension and of the challenge liquid effluent within the biowaste treatment system. Determine the concentration of each suspension by preparing serial dilutions of these samples. Plate in triplicate 0.1 or one ml of each diluent sample on Trypticase Soy Agar.

(6) Operate the biowaste treatment system through a normal cycle. Record operating temperatures, pressures, and quantities or flow rates.

(7) Aseptically collect a minimum of three - 100 ml samples of final-treated effluent. Prepare serial dilutions and plate in triplicate 0.1 or one ml samples on Trypticase Soy Agar. The remaining original sample and each diluted sample are to be filtered through a 0.2 μ m size membrane filter. Aseptically place the membrane filters on Trypticase Soy Agar. Incubate culture plates at 37° C for a total of 72 hours. Culture plates are to be examined at 24, 48, and 72 hours for growth.

(8) Three replicate tests are to be conducted.

c. Test Criterion

All replicate test samples from liquid effluent following the sterilization cycle are to have no viable growth.

V. Packaging and Shipping of Recombinant DNA Materials

Federal regulations and carrier tariffs have been promulgated to ensure the safe transport of hazardous biological materials. The NIH Guidelines specify that all organisms containing recombinant DNA molecules will be packaged and shipped in containers that meet the requirements of these regulations and carrier tariffs.

A. Instruction for the Packaging of Host and Vector Organisms Containing Recombinant DNA Molecules

1. Volume less than 50 ml

Place the material in a securely closed, watertight container [primary container (test tube, vial, etc.)]. Place the primary container in a second, durable watertight container (secondary container). Several primary containers may be enclosed in a single secondary container if the total volume of all the primary containers so enclosed does not exceed 50 ml. Fill the space at the top, bottom, and sides between the primary and secondary containers with sufficient nonparticulate absorbent material to absorb the entire contents of the primary container(s) in case of breakage or leakage. Then enclose each set of primary and secondary containers in an outer shipping container constructed of corrugated fiberboard, cardboard, wood, or other material of equivalent strength.

If dry ice is used as a refrigerant, it must be placed outside the secondary container(s).

Descriptions of this packaging method are given in the following table.

DESCRIPTION OF PACKAGES FOR MATERIAL IN VOLUMES LESS THAN 50 ml.

Volume (ml)	Primary Container	Packing	Secondary Container	Packing	Outer Shipping ^d Container
15 max.	Sealed vial(s) or small glass test tube, screw cap* or stopper, taped	a	Metal can 1" diam. x 7" O.D. metal screw cap	None Required	Fiberbody; metal screw cap, top and bottom; 1½" diam. x 7 to 7½" O.D.
50 or less	One 20 x 150 mm test tube, taped* stopper or multiple small vials	a	Metal can 2½" diam. x 6½" high O.D. screw cap	None Required	Fiberbody; metal screw cap, top and bottom; 3¼" diam. x 7 to 7½" O.D.
50 or less	Plastic* screw cap* bottle or Pyrex glass with skirt rubber stopper	a	Metal can 2½" diam. x 6½" high O.D. screw cap	None Required	Fiberbody; metal screw cap, top and bottom; 3¼" diam. x 7 to 7½" O.D.
50 or less	Multiple watertight vials or * tubes, taped stoppers	a	One or more friction-seal tin cans ^b 306 x 400 or larger	c	Fiberboard box

*The flexibility of the plastic bottle requires that a stopper or screw cap be secured in place by adhesive tape. The usual equivalent-size glass flat-sided prescription bottle is too fragile for use. For air transport, all stoppers, corks, and caps on primary containers must be secured in place with wire, tape, or other means, and all screw-capped containers of unfrozen liquid must be placed in 5 or 6 mil polyvinyl tubing heat-sealed at both ends to prevent atmospheric decompression that may result in leakage past the screw cap.

O.D. = outside dimensions.

a Nonparticulate absorbent material at top, bottom and sides that will completely absorb contents of the primary container(s).

b 610 x 708 and 804 x 908 are trade designations for outside dimensions of 6-10/16 inches diameter x 7-8/16" height, and 8-4/16" x 9-8/16" .

c None required, but with the 306 x 400 cans or larger cans use sufficient nonparticulate shock-absorbent material to prevent rattling.

d If materials are to be refrigerated, it is recommended that an overpack be used to contain the refrigerant and the secured (original) outer shipping container. A leak proof outer container must be used for water ice. If dry ice is used the outer container must permit release of carbon dioxide. Interior supports must be provided to hold the container(s) in the original position(s) after wet or dry ice has dissipated.

2. Volumes of 50 ml or Greater

Place the material in a securely closed, watertight container (primary container). Enclose this container in a second, durable watertight container (secondary container). Single primary containers are not to contain more than 500 ml of material. However, two or more primary containers whose combined volumes do not exceed 500 ml may be placed in a single secondary container. Fill the space at the top, bottom, and sides between the primary and secondary containers with sufficient non-particulate absorbent material to absorb the entire contents of the primary container(s) in case of breakage or leakage. Then enclose each set of primary and secondary containers in an outer shipping container constructed of corrugated fiberboard, cardboard, wood, or other material of equivalent strength. Also place a shock-absorbent material, in volume at least equal to that of the absorbent material, between the primary and secondary containers at the top, bottom, and sides, between the secondary container and the outer shipping container. (The maximum amount of materials that may be enclosed within a single outer shipping container should not exceed 4000 ml.)

If dry ice is used as a refrigerant, it must be placed outside the secondary container(s). If dry ice is used between the secondary container and the outer shipping container, the shock-absorbent material is to be placed so that the secondary container does not become loose inside the outer shipping container as the dry ice sublimates.

Descriptions of packages that comply with the regulations of the Department of Transportation (DOT) are given in the following table.

B. Labeling of Packages Containing Host and Vector Organisms that Contain Recombinant DNA Molecules

Material data forms, letters, and other information identifying or describing the material should be placed around the outside of the secondary container. The label for Etiologic Agents/Biomedical Material must be affixed to the outer shipping container. This label is described in the Code of Federal Regulations, paragraph (c) (4) of 42 CFR 72.25.

DESCRIPTION OF PACKAGES FOR MATERIAL IN VOLUMES OF 50 ml OR GREATER

Volume (ml)	Primary Container	Packing	Secondary Container	Packing		Outer Shipping Container	
				With Refrigerant	Without Refrigerant	With Refrigerant	Without Refrigerant
51 to 100 ml	Plastic* or Pyrex glass screw cap* bottle, rubber or skirt rubber stopper, taped*	a	Consists of metal container & outer container specified in Table 1	Styrofoam box shock-absorbent insulation	c	Fiberboard box closely fitting the styrofoam box, taped shut	Corrugated fiberboard or cardboard box, taped shut
100 max.	One 100 ml plastic* screw cap* narrow neck bottle or Pyrex glass, taped*	a	No. 3 crimp seal tin can 404 x 700 or a 1-gallon friction-seal tin can, 610 x 708, top soldered or clipped at 4 points b	Styrofoam box shock-absorbent insulation	c	Fiberboard box closely fitting the styrofoam box, taped shut	V3C cardboard box PS3 type, 9-3/16" x 9-3/16" x 11 1/4" high O.D. taped shut with 3" type PS3 tape
200 max.	Two 100 ml plastic* screw cap* bottles or Pyrex glass, taped	a	No. 3 crimp seal tin can 404 x 700 or a 1-gallon friction-seal tin can, 610 x 708, top soldered or clipped at 4 points b	Styrofoam box shock-absorbent insulation	c	Fiberboard box closely fitting the styrofoam box, taped shut	V3C cardboard box PS3 type, 9-3/16" x 9-3/16" x 11 1/4" high O.D. taped shut with 3" type PS3 tape
250 max.	One 250 ml plastic* narrow mouth screw cap* bottle or Pyrex glass skirted rubber stopper, taped*	a	No. 3 crimp seal tin can 404 x 700 or a 1-gallon friction-seal tin can, 610 x 708, top soldered or clipped at 4 points b	Styrofoam box shock-absorbent insulation	c	Fiberboard box closely fitting the styrofoam box, taped shut	V3C cardboard box PS3 type, 9-3/16" x 9-3/16" x 11 1/4" high O.D. taped shut with 3" type PS3 tape
500 max.	Two 250 ml plastic* screw cap* bottles or Pyrex glass bottles, taped*	a	2-gallon friction-seal tin can, 804 x 908, top soldered or clipped at 4 points b	Styrofoam box shock-absorbent insulation	c	Fiberboard box closely fitting the styrofoam box, taped shut	V3C cardboard box 12 1/2" x 12 1/2" x 10-3/16" high O.D. taped shut with 3" wide PS3 tape
500 max.	500 ml Pyrex glass bottle, rubber-skirt stopper, taped, or 500 ml plastic* bottle, narrow or wide mouth, screw cap*, taped	a	No. 12 crimp seal tin can 603 x 810 2-gallon friction-seal tin can, 804 x 908, top soldered or clipped at 4 points b	Styrofoam box shock-absorbent insulation	c	Fiberboard box closely fitting the styrofoam box, taped shut	V3C cardboard box 12 1/2" x 12 1/2" x 10-3/16" high O.D. taped shut with 3" wide PS3 tape. For the No. 12 can a cardboard box is ok taped shut

*The flexibility of the plastic bottle requires that a stopper or screw cap be secured in place by adhesive tape. The usual equivalent-size glass flat-sided prescription bottle is too fragile for use. For air transport, all stoppers, corks, and caps on primary containers must be secured in place with wire, tape, or other means, and all screw-capped containers of unfrozen liquid must be placed in 5 or 6 mil polyvinyl tubing heat-sealed at both ends to prevent atmospheric decompression that may result in leakage past the screw cap.

O.D. = outside dimensions.

a Nonparticulate absorbent material at top, bottom and sides that will completely absorb contents of the primary container(s).

b 610 x 708 and 804 x 908 are trade designations for outside dimensions of 6-10/16 inches diameter x 7-8/16" height, and 8-4/16" x 9-8/16".

c Shock absorbent material, in volume at least equal to that between the primary and secondary container(s), at the top, bottom, and sides between the secondary container and the outer shipping container. The shock absorbent material shall be so placed that the secondary container(s) does not become loose inside the outer shipping container as the water ice or dry ice is dissipated.

In addition, if the materials to be shipped contain any portion of a plant pest (plant pathogens), which is so defined by the Department of Agriculture (USDA), the outer shipping container must have affixed to it the shipping label furnished by the USDA as part of the General, Courtesy, or Special Permits required for research with and shipment of such agents.

C. Additional Shipping Requirements and Limitations for Host and Vector Organisms that Contain Recombinant DNA Molecules

1. Domestic Transportation

a. Quantities less than 50 ml

The Air Transport Association's (ATA's) Restricted Articles Tariff 6-D, which specifies transportation requirements for hazardous materials shipped by air, was amended June 25, 1977. One of the provisions of this amended tariff, effective September 1, 1977, is that a large Shipper's Certificate must be used on domestic as well as international air shipments of etiologic agents and other hazardous materials.

This new requirement has prompted a review of applicable regulations and tariffs for alternatives to the use of this large Shipper's Certificate on small (less than 50 ml) shipments of etiologic agents sent by mail and airmail. The following statement has been reviewed with the U. S. Department of Transportation, the U. S. Postal Service, and the ATA, and it is consistent with the requirements of each of these agencies. In place of the large certificate, this statement should be used on all domestic shipments of organisms containing recombinant DNA molecules less than 50 ml in volume.

NOTICE TO CARRIER

This package contains LESS THAN 50 ml OF AN ETIOLOGIC AGENT, N.O.S., is packaged and labeled in accordance with the U.S. Public Health Service Interstate Quarantine Regulations (42 CFR, Section 72.25 (c) (1) and (4)), and MEETS ALL REQUIREMENTS FOR SHIPMENT BY MAIL AND ON PASSENGER AIRCRAFT.

This shipment is EXEMPTED FROM ATA RESTRICTED ARTICLES TARIFF 6-D (see General Requirements 386 (d) (1)) and from DOT HAZARDOUS MATERIALS REGULATIONS (see 49 CFR, Section 173.386 (d) (3)). SHIPPER'S CERTIFICATES, SHIPPING PAPERS, AND OTHER DOCUMENTATION OR LABELING ARE NOT REQUIRED.

Date

Signature of Shipper

Address

b. Quantities equal to or greater than 50 ml

Shipments of materials exceeding 50 ml in volume are restricted, by DOT regulations, to transport by cargo-only aircraft. When the volume of a single primary container exceeds the 50 ml limitation, this restriction must be indicated on a Shipper's Certificate. An appropriate certificate is shown on the following page.

When dry ice is used as a refrigerant, an ORA, Group A, dry ice label should be affixed to the outer shipping container. The amount of dry ice used and the date packed should be designated on the label.

SHIPPER'S CERTIFICATION FOR RESTRICTED ARTICLES
(excluding radioactive materials)

Two completed and signed copies of this certification shall be handed to the carrier. (Use block letters.)

WARNING: Failure to comply in all respects with the applicable regulations of the Department of Transportation, 49-CFR, CAB 82 and, for international shipments, the IATA Restricted Articles Regulations may be a breach of the applicable law, subject to legal penalties. This certification shall in no circumstance be signed by an IATA Cargo Agent or a consolidator for international shipments.

This shipment is within the limitations prescribed for: *(mark one)*

passenger aircraft cargo-only aircraft

Number of Packages	Article Number (int'l only See Section (V IATA RAR))	Proper Shipping Name of Articles as shown in Title 49 CFR, CAB 82 Tariff 6-D and (for int'l shipments) the IATA Restricted Articles Regulations. Specify each article separately. Technical name must follow in parenthesis, the proper shipping name for N.O.S. items.	Class	IATA Packing Note No. Applied (int'l only)	Net Quantity per Package	Flash Point (Closed cup) For Flammable Liquids	
						°C	°F

Special Handling Information:

I hereby certify that the contents of this consignment are fully and accurately described above by Proper Shipping Name and are classified, packed, marked, labelled and in proper condition for carriage by air according to applicable national governmental regulations, and for International Shipments the current IATA Restricted Articles Regulations.

Name and full address of Shipper		Name and title of person signing Certification	
Date		Signature of the Shipper (see WARNING above)	
Air Waybill No.*	Airport of Departure*	Airport of Destination*	

*This box is optional for completion by issuing carrier.

Form 776

2. International Transportation

In addition to the packaging and labeling requirements of the regulations previously cited, international shipments of these materials must have one or more of the following documents—depending on the country of destination:

- Parcel Post Customs Declaration (PS 2966) tag.
- Parcel Post Customs Declaration (PS 2966-A) label.
- International Parcel Post—Instructions Given by Sender (POD 2922) label.
- Dispatch Note (POD 2972) tag.
- Shipper's Certificate specified in the current International Air Transport Association Tariff.

Individual country requirements are listed in "International Postage Rates and Fees" (USPO Publication 51).

D. Packaging of Recombinant DNA Molecules

Recombinant DNA molecules shall be packaged to withstand leakage of contents, shocks, pressure changes, and other conditions incident to ordinary handling in transportation.

TO BE REVISED
ROLES AND RESPONSIBILITIES

Section VI - A. pages 181 - 190

VI. Roles and Responsibilities

A. The Institutional Biosafety Committee

Research involving biological agents capable of causing disease or illness in man has been conducted for many decades. The protection of laboratory workers and the general environment from harm has been largely left up to the scientists directly involved in such research. This has apparently worked well, since there have been no major incidents of spread of disease outside of the laboratory and the number of laboratory-acquired illnesses have been relatively few. However, with the greatly intensified research now going on with known hazardous and potentially hazardous biological agents, it is prudent to provide some level of institutional oversight for such research activities. The "NIH Guidelines for Recombinant DNA Research" require that such oversight be provided by an Institutional Biosafety Committee (IBC) that serves the entire institution for research involving the application of recombinant DNA techniques. The committee is to act on behalf of the institution.

1. Functions

The IBC should have three principal functions:

- Advise the institution on development and implementation of policies for the safe conduct of research involving recombinant DNA molecules.
- Review and oversee all recombinant DNA projects.
- Advise the institution and NIH whether proposals for research involving recombinant DNA molecules comply with "NIH Guidelines for Recombinant DNA Research" and institutional policies.

The "NIH Guidelines for Recombinant DNA Research" outline a number of duties and responsibilities of the IBC. These are summarized in the following table, which is arranged in a manner to give emphasis to the interrelationships among the institution, the principal investigator, the IBC, and the NIH in carrying out duties and responsibilities pertaining to the development and implementation of the "NIH Guidelines for Recombinant DNA Research."

2. Organization and Membership

Because of the different administrative schemes used by institutions engaged in recombinant DNA research, it is impossible to make specific suggestions as to the best method of establishing the IBC. An important basic principle to follow, however, is that the IBC should be established by the highest administrator in the institution. This establishes the institution-wide interests of the committee; involves top administration in the deliberations of the committee; and assures close communication between the committee and institutional officers.

The size of the committee will be somewhat dependent on the size and complexity of the institution; but, in general, committees of 9 to 15 members should be able to function quite satisfactorily. No committee should have less than five members. There should be provision for rotation of membership. This can be done by limiting the tenure of membership to three, four, or five years. Less than three years may be too short a time to develop the full effectiveness of the members and more than five years would seem to place too much of a burden on the members.

The qualifications of the individuals to serve on the IBC need careful consideration. The membership should include individuals from a diversity of scientific disciplines relevant to recombinant DNA technology. The fields could include microbiology, virology, molecular biology, epidemiology, and ecology. The membership should also include individuals who are knowledgeable about laboratory safety and engineering principles.

**TABULATION OF DUTIES AND RESPONSIBILITIES
PERTAINING TO THE DEVELOPMENT AND IMPLEMENTATION
OF NIH GUIDELINES FOR RECOMBINANT DNA RESEARCH**

DUTIES	RESPONSIBILITIES	
	Institution	National Institutes of Health
	IAd : Institutional Administration BSO: Biological Safety Officer IBC : Institutional Biosafety Committee PI : Principal Investigator	OD : Office of the Director, NIH ORDA: Office of Recombinant DNA Activities, NIGMS, NIH C : Other NIH Components RAC : Recombinant DNA Molecule Program Advisory Committee
A. Developing NIH Guidelines and Policy		
1. Prepare Guidelines and establish policy. Specify permissible, exempt and prohibited experiments.	PI : Recommend to ORDA or OD IBC : Recommend to ORDA or OD	ORDA: Support RAC RAC : Recommend to OD OD : Take action after appropriate notice and opportunity for public comment
2. Revise and amend Guidelines.	PI : Recommend to ORDA IBC : Recommend to ORDA	RAC : Recommend to OD ORDA: Recommend to OD OD : Take action after appropriate notice and opportunity for public comment
3. Promulgate and amend a list of classes of experiments to be exempt from Guidelines.	PI : Recommend to ORDA IBC : Recommend to ORDA	RAC : Recommend to OD ORDA: Recommend to OD OD : Take action after appropriate notice and opportunity for public comment
4. Designate classification of agents (on basis of hazard) for purposes of Guidelines.		RAC : Recommend to OD OD : Take action
5. Certify and de-certify host-vector systems. Maintain public listing.	PI : Submit information to ORDA	RAC : Advise OD ORDA: Advise OD OD : Take action after appropriate notice and opportunity for public comment ORDA: Administer
6. Permit exceptions to prohibitions.	PI : Notify IBC and request action of ORDA	RAC : Advise OD ORDA: Advise OD OD : Take action after appropriate notice and opportunity for public comment
7. Interpret Guidelines.	PI : Request action of ORDA IBC : Request action of ORDA	RAC : Advise OD Advise ORDA ORDA: Take action or recommend to OD OD : Take action
8. Publish <u>Recombinant DNA Technical Bulletin</u> .	PI : Provide information to ORDA IBC : Provide information to ORDA	ORDA: Take action
9. Ensure limitation of disclosure of confidential or proprietary information.	PI : Comply IBC : Comply IAd : Comply	NIH : Comply
10. Submit application for patent if information is regarded as proprietary.	PI : Initiate action IAd : Review and take action if desired	NIH : Respect proprietary information

B. Establishing an Institutional Recombinant DNA Research Program		
1. Establish an Institutional Biosafety Committee. Select and report names and qualifications to ORDA. Update information annually.	IAd : Take action	ORDA: Review and advise IAd
2. Appoint a Biological Safety Officer for projects at the P3 and P4 containment levels.	IAd : Take action	ORDA: Advise
3. Establish Institutional policies and practices to be followed in conforming with NIH Guidelines.	IBC : Recommend to IAd IAd : Take action	ORDA: Advise
4. Keep minutes of IBC meetings.	IBC : Take action	
5. Submit proposals to conduct recombinant DNA research to IBC.	PI : Take action	
6. Make independent determination of the required levels of physical and biological containment in accordance with NIH Guidelines.	PI : Take action IBC : Take action	ORDA: Take action
7. Select appropriate microbiological practices and laboratory techniques to be used in recombinant DNA research.	PI : Take action BSO: Advise re: P3 and P4	ORDA: Advise
8. For each recombinant DNA research project proposed by a PI, determine that facilities, procedures, and practices and the training and expertise of personnel are in compliance with NIH Guidelines.	BSO: Advise re: P3 and P4 IBC : Take action	ORDA: Take action C : Certify P4 facilities
9. Review each project and establish, if necessary, a medical surveillance program.	IBC : Recommend to IAd IAd : Take action	ORDA: Advise
10. Establish emergency plans.	PI : Recommend BSO: Recommend re: P3 and P4 IBC : Take action	ORDA: Advise
11. Execute an MUA (or submit equivalent information in case of non-NIH supported project to accomplish registration) and submit to NIH after approval by IBC. Each application to the NIH for a project that involves experiments subject to the NIH Guidelines must be accompanied by an MUA. For ongoing projects that will be introducing new recombinant DNA experiments, an MUA should be submitted to NIH within 30 days of approval by the IBC.	PI : Initiate BSO: Advise re: P3 and P4 IBC : Review and approve IAd : Take action	ORDA: Take action C : Transmit MUA to ORDA for review and approval

C. Conducting the Research Program		
1. Initiate no recombinant DNA research until it has been approved by the IBC.	PI : Take action	
2. Comply fully with Guidelines in carrying out research.	PI : Take action	ORDA: Advise
3. Adhere to IBC-approved emergency plans.	PI : Take action BSO: Advise re: P3 and P4	
4. Comply with shipping requirements for recombinant DNA molecules.	PI : Take action BSO: Advise re: P3 and P4	ORDA: Advise
5. Inform laboratory staff of approved safety protocols that describe the potential biohazards and precautions to be taken.	PI : Take action BSO: Advise re: P3 and P4	
6. Instruct and train staff in the practices and techniques required to ensure safety, and in the procedures for dealing with accidents.	PI : Take action BSO: Advise re: P3 and P4	ORDA: Advise
7. Inform staff of the reasons and provisions for any advised or requested medical practices, vaccinations, or serum collection.	PI : Take action	
8. Execute a revised MUA for projects involving substantial changes in protocol and submit to NIH within 30 days of approval by IBC.	PI : Initiate BSO: Advise re: P3 and P4 IBC: Review and approve IAd: Take action	ORDA: Take action; notify IAd when IBC-approved MUA does not conform to NIH Guidelines
9. Make changes to IBC-approved MUA to conform to ORDA review requirements.	PI : Take action IAd: Inform PI	ORDA: Advise
10. Request reductions in containment levels for purified DNA and characterized clones.	PI : Initiate IBC: Take action except for the following cases that are to be referred to ORDA (i) those involving primate DNA, (ii) requests for more than a single-step reduction, and (iii) requests for lowering containment levels below P1 and HV1. Notify ORDA of action or submit request to ORDA for action.	RAC : Advise ORDA ORDA: Take action

D. Supervising and Appraising of Safe Conduct of Research		
1. Supervise safe conduct of research.	PI : Take action	
2. Correct work errors and conditions that may result in the release of recombinant DNA materials.	PI : Take action	
3. Report promptly to the IBC and ORDA any problems or violations of the Guidelines.	PI : Take action IBC : Take action	ORDA: Review and recommend to OD OD : Take action
4. Investigate and report in writing to the IBC, ORDA, and the BSO (where applicable) any significant problems pertaining to operation and implementation of biological and physical containment safety practices and procedures.	PI : Take action BSO: Review and Advise re: P3 and P4 IBC : Review and take action	ORDA: Review, take action or recommend to OD OD : Take action as required
5. Provide advice on laboratory security.	BSO: Take action re: P3 and P4	ORDA: Advise

E. Monitoring of Safe Conduct of Research		
1. Ensure compliance of recombinant DNA projects with the procedures and standards of the NIH Guidelines.	IAd : Take action	ORDA: Advise
2. Review periodically recombinant DNA research being conducted at the institution.	IBC : Take action	
3. Ensure, through periodic inspection, that laboratory safety standards are rigorously followed (P3 and P4 containment levels).	BSO: Take action re: P3 and P4	C : Make on-site inspection at P4 facilities
4. Investigate and report in writing to ORDA and the IBC any serious or extended illness of a worker; any incident causing serious exposure to personnel or danger of environmental contamination; and any accident that involves inoculation of recombinant DNA material through the skin, by ingestion, or probable inhalation.	PI : Take action IBC : Review and take action	ORDA: Review, take action or recommend to OD OD : Take action as required
5. Ensure integrity of physical containment.	PI : Take action	
6. Ensure integrity of biological containment (e.g., genotypic and phenotypic characteristics, purity, etc.)	PI : Take action	
7. Oversee implementation of the NIH Guidelines.	IBC : Take action IAd : Take action	OD : Take action

Appointment of a nondoctoral person from a laboratory technical staff can provide a useful perspective of the working laboratory setting.

In addition to possessing the professional competence necessary to assess and review specific activities and facilities, the committee should possess, or have available to it, the competence to determine the acceptability of its findings in terms of applicable laws, regulations, standards of practices, community attitudes, and health and environmental considerations.

Individually, the members should be recognized by their colleagues as capable scientists and persons of good judgment. The members should each have a personal commitment to laboratory safety in general and biosafety in particular.

Any institution that has need for an IBC must recognize that there may be legitimate concern in the adjacent community about whether adequate safeguards are being taken at the institution to protect the general environment and community from potentially hazardous materials. How this concern might best be dealt with will vary from one community to another, but the institution should not ignore this concern. One suggestion is that community individuals with either statutory or political responsibility for the health and well-being of the residents of the community be invited to attend the meetings of the IBC and to participate in the deliberations of the committee when matters of concern to the public are under consideration. The minutes of the committee meetings could be shared with these individuals upon request. Another suggestion would be to appoint such an individual to serve as a member of the committee.

3. General Recommendations

An independent assessment of the physical and biological containment levels for each recombinant DNA project and the analysis of requests for single step reductions in containment levels for experiments with purified DNA and characterized clones require considerable scientific

expertise, as well as scientific judgment. Not all IBCs will have among its members sufficient background and experience to deal thoroughly with the technical aspects of all project proposals that come to its attention. Advice and consultation from institutional scientists who are not members of the IBC may be desirable and necessary. It is suggested, therefore, that the IBC identify institutional scientists who could serve as consultants to the committee. The use of such consultants in supporting the work of the committee is to be encouraged.

Many institutions have established environmental health and safety programs that deal with the broad area of laboratory safety. These programs are likely to have resources and capabilities that could be used to support the work of the IBC. For example, such programs may be well equipped to perform surveillance and monitoring of safety practices; review the integrity of safety equipment and facilities; investigate problems pertaining to operations and implementation of physical containment safeguards; investigate accidents causing serious exposure to personnel or danger of environmental contamination; assist principal investigators in the selection of appropriate safety practices and equipment and in the preparation of MUAs; and develop emergency procedures. These programs could also provide staff support to the IBC. It is suggested, therefore, that the IBC utilize the resources and capabilities of environmental health and safety programs, where appropriate, in the support of the work of the committee. This arrangement could be most beneficial in areas dealing with operational responsibilities of the committee such as those listed above.

The work of the committee should be conducted in a deliberate but timely manner. The process by which the committee functions should be well defined and known by all institutional scientists who may wish to use recombinant DNA techniques in their research. This suggests that the procedures to be used by the committee and the logistics for maintaining communication with the principal investigator and other interested persons

should be documented. This will facilitate cooperation and will minimize difficulties in conducting the work of the committee.

The following steps and procedures are offered for an IBC for reviewing and approving MUAs:

- The principal investigator should submit the proposed MUA to the executive secretary or the chairman of the IBC. It would be preferable to submit the document to the executive secretary, who would assign an identification number for administrative control during the review process and for future reference. The executive secretary would then initiate the review process.

- The MUA should then be transmitted to committee members. The members who receive the MUA may vary, depending on the workload, the proposed level of physical containment, and the committee review process. All committee members, however, should receive copies of any MUA for which the principal investigator has determined that a P3 or P4 level of physical containment would be required.

- It may be advisable to establish a scientific subcommittee that would be responsible for conducting an independent assessment of the physical and biological containment levels appropriate for a submitted MUA. This subcommittee should have sufficient flexibility to seek advice from other scientists, as required. After it has completed its assessment, the subcommittee should also be encouraged to discuss with the principal investigator any differences it has with respect to the initial assessment performed by the principal investigator. This may facilitate an early modification to the MUA, if required, prior to formal committee action.

- The subcommittee should submit its recommendations to the IBC within one month after receipt of the MUA, or sooner, if possible.

- Following IBC approval of the assessment conducted by the subcommittee, the IBC should determine that the required safeguards and expertise of the project staff for the assessed level of physical containment are available. Such a determination may require a visit to the laboratory in which the project is to be conducted. Visits for projects requiring P3 or P4 levels of physical containment should be coordinated by the biological safety officer. The professional staff of the institution's environmental health and safety program may be helpful in performing this task for the committee. Once this review has been completed, the committee would be in a position to make a final decision on the MUA.

- The final decision on the MUA should be transmitted, in writing, to the principal investigator. An approved copy of the MUA would serve this purpose. The committee should also initiate the submittal process for notifying NIH of the IBC approval action.

- The IBC should develop a mechanism for reviewing periodically all projects having approved MUAs. This should be done to determine if new data or revised guidelines for assessing potential hazards warrant the revision of the MUA. Also, the IBC should be advised as to whether the project has been completed, temporarily stopped, or terminated for any reason.

B. The Biological Safety Officer

The "NIH Guidelines for Recombinant DNA Research" require that institutions engaged in recombinant DNA research at the P3 or P4 physical containment levels appoint a person who will serve as a biological safety officer. The following duties will be among those to be performed by the biological safety officer:

- Provide technical advice to the principal investigator and IBC on research safety procedures.
- Provide advice on laboratory security.
- Develop emergency plans for dealing with accidental spills and personnel contamination; and investigate recombinant DNA research laboratory accidents.
- Ensure through periodic inspections that laboratory standards are rigorously followed.
- Serve as a member of the IBC.

The principal function of the biological safety officer should be to advise the principal investigator, the IBC and the laboratory worker concerning the most appropriate safety practice that will assure the safe conduct of recombinant DNA research.

Depending on the nature and extent of the institution's recombinant DNA programs, the biological safety officer may be a full-time position, or the duties may be assigned to an individual who has other responsibilities. Where the institution has a comprehensive environmental health and safety program that includes expertise in biological safety, it would be useful to select the individual from the program's professional staff. This would ensure effective collaboration with other safety professionals and would allow all elements of a laboratory safety program to be carried out in a unified manner. Where this expertise does not exist, the appointed individual should interact closely with the basic safety group of the institution. If the position is to be full-time, consideration should be given to assigning this position to the basic health and safety program, rather than creating a separate program for biosafety in a different administrative setting.

The qualifications of the individual need careful consideration. Familiarity with the laboratory setting and the basic scientific techniques and manipulations common to biomedical research is important. Ideally, the person should have experience in working within a biomedical research setting. A technical background in basic microbiology and knowledge of biological safety techniques and practices, containment equipment, and engineering principles pertaining to the design and operation of facility safeguards are also important. The individual should be able to communicate effectively with technical, administrative, and support personnel.

Additional duties and responsibilities may be assigned to the biological safety officer. These will depend on the magnitude and complexity of the institution's recombinant DNA program, whether the position is full- or part-time, the relationship of the position to the institution's environmental health and safety program, and the qualifications of the individual. The following are examples of additional duties and responsibilities:

- Provide special laboratory safety training.
- Serve as a liaison with NIH and other research organizations on matters pertaining to laboratory safety.
- Conduct or supervise all testing programs designed to demonstrate the integrity of containment equipment and facility safeguards.
- Supervise emergency decontamination measures.
- Maintain a safety library of reference publications and training materials.
- Provide guidance and assistance concerning the packaging and shipping of recombinant DNA materials.

The full extent of these activities, if they were required, could not be carried out by a single person. These activities may be distributed, however, among the staff of the institution's environmental health and safety program. In such a case, the biological safety officer should be responsible for the management, supervision or coordination of these activities.

At the present time few individuals possess the experience and expertise to function independently as biological safety officers. Training, discussion of safety matters with other safety professionals, and on-the-job experience will help alleviate these shortcomings. Also, NIH is initiating a comprehensive training program on the practices and procedures for the control of biohazards in the research laboratory. The primary objective of this training effort will be to give detailed instruction on the duties of the biological safety officer.

C. Emergency Procedures

Safety is an intrinsic part of each laboratory operation; work is planned so that exposure to potentially hazardous agents will not occur. In spite of this, accidents that create hazards do occur. These may involve spills of potentially hazardous agents in the open laboratory. Also, failure of important equipment and facility safeguards may place the laboratory worker at a high risk of accidental exposure. Likelihood of severe injury or infection can be reduced if plans for emergencies are established and are well known to all who need to know. For this reason, the "NIH Guidelines for Recombinant DNA Research" require the preparation of emergency plans for laboratories involved in this research.

It is not possible to recommend a single plan of action that would be applicable in all situations. The following basic principles, however, may be useful in developing specific procedures for dealing with accidental spills of potentially hazardous materials in the open laboratory.

- Get everyone out of the affected area.
- Do not reenter until the extent of the hazard is determined.
- Determine the necessity for treating persons exposed to the potentially hazardous materials.
- Decontaminate the affected area (see Section II,E,6).

For emergencies involving the failure of equipment or facility safeguards, the most important action should be to stop work with potentially hazardous materials and to safely contain these materials until corrective action has been taken.

An important principle in any emergency situation is that attention to the immediate personal danger overrides maintenance of containment. Potentially hazardous materials should be safely contained insofar as this is compatible with this principle. In cases of serious injury or sudden illness, the principal investigator should determine whether to override containment procedures.

Emergency plans for dealing with fire, explosion, and natural disaster are also important. Most institutions have plans developed for such situations, and extensive literature is available on this subject. The

following references are examples of such literature:

Manufacturing Chemists Association. 1972. Guide for safety in the chemical laboratory. Van Nostrand Reinhold Company, New York, NY.

McKinnon, G.P., and K. Tower, eds. 1976. Fire protection handbook. National Fire Protection Association, Boston, MA.

Morse, G.P., and R.F. Morse. 1974. Protecting the health care facility. The Williams and Wilkins Company, Baltimore, MD.

Steere, N.V., ed. 1971. Handbook of laboratory safety. The Chemical Rubber Company, Cleveland, OH.

Because all emergencies cannot be anticipated in advance, the National Institutes of Health and the Center for Disease Control are available to provide consultation and direct assistance, if necessary, to assist institutions in the management of specific emergency situations. Assistance can be obtained by contacting one of the following offices:

- Office of Research Safety
National Cancer Institute
National Institutes of Health
(301) 496-1862
- Environmental Safety Branch
Division of Research Services
National Institutes of Health
(301) 496-6034
- Office of Biosafety
Center for Disease Control
(404) 633-3311, Ext. 3883

D. Medical Surveillance

The "NIH Guidelines for Recombinant DNA Research" state that it is the responsibility of the Institution to "determine, in connection with each project, the necessity for medical surveillance of recombinant DNA research personnel before, during, and after their involvement in this research." The Guidelines further require the principal investigator to investigate and report "in writing to ORDA and the IBC any serious or extended illnesses of a worker or any accident that results in (i) inoculation of recombinant DNA materials through cutaneous penetration, (ii) ingestion of recombinant DNA materials, (iii) probable inhalation of recombinant DNA materials following gross aerosolization, or (iv) any incident causing serious exposure to personnel or danger of environmental contamination." These activities constitute the minimum requirements for a medical surveillance program for recombinant DNA research. The Guidelines also recommend that the medical surveillance program provide for collection and maintenance of serum samples and for the immunization of all workers who may work with known pathogens for which an effective vaccine is available. This section provides further guidance to institutions on activities that may be considered for inclusion in a medical surveillance program.

The extent of any medical surveillance program will vary greatly, depending upon the nature and size of the research project and the available medical facilities. For example, a comprehensive medical surveillance program including preassignment and periodic physical and other medical examinations may not be appropriate for laboratory workers involved in research requiring P1 and P2 levels of physical containment, whereas such a program may be advisable for workers engaged in certain research projects requiring P3 physical containment and would be recommended for workers engaged in research requiring P4 physical containment.

The objective of this review is to provide pertinent information and call attention to various functions related to medical surveillance that are recommended for consideration, insofar as they are applicable

to recombinant DNA research. These recommendations are intended to supplement normal pre-employment medical examinations that determine suitability of a prospective employee to a particular job situation.

1. Serum Collection Program

The collection and maintenance of serum specimens from laboratory workers engaged in research with potentially hazardous organisms will provide the potential for monitoring serological changes that may result from the employee's work experience. Serological conversion warns that accidents, procedures, or equipment have caused significant exposure of personnel to research material. A report of seroconversion is a clear signal calling for examination, identification, and revision of the laboratory procedures that have exposed the laboratory worker.

In order to establish a meaningful serum collection program, sera should be obtained from all personnel who may be potentially exposed to potentially hazardous organisms. This includes personnel handling potentially hazardous organisms as well as personnel assigned to areas where these materials are handled.

The principal investigator should arrange with the institution's medical service to establish a schedule to obtain serum specimens from each laboratory worker prior to the time that work with potentially hazardous organisms is initiated, at yearly intervals as a minimum frequency thereafter, and prior to termination of employment. In addition, serum specimens should be obtained immediately following an overt exposure and at an appropriate time after such an exposure. When agents which are known to be capable of producing a serological response are used in the research, it is advisable to prepare two samples each time serum is collected. One sample should be evaluated for antibodies and the other stored in a freezer for future reference.

2. Immunizations

Immunization is generally recommended for laboratory workers who will be engaged in research with infectious organisms for which an effective vaccine is available. At some institutions, prior immunization may be required for certain positions as a condition of employment. Where immunizations are required, evidence of antibody response should be demonstrated, whenever possible, before an employee begins to work with infectious organisms. Detailed information on vaccines and general recommendations for the immunization of laboratory workers can be found in "Lab Safety at the Center for Disease Control" U. S. Department of Health, Education, and Welfare, Public Health Service, HEW Publication No. CDC 77-8118.

3. Reporting and Investigating of Accidents and Illnesses

If an organism containing recombinant DNA molecules were to acquire the capacity to infect and cause disease in man, the first evidence of this potential may be demonstrated by a laboratory-acquired infection. For this reason it is important to investigate any serious, unusual, or extended illness of a laboratory worker engaged in recombinant DNA research or any accident that involves inoculation of organisms containing recombinant DNA molecules through the skin, by ingestion, or probable inhalation. A finding that infection is associated with recombinant DNA research will provide sufficient warning for evaluation of hazards and initiation of additional precautions to protect the general public, if such protection is necessary.

Prompt reporting of accidents involving overt exposures is essential (See Section VI, C, "Emergency Procedures"). The laboratory worker involved with such an occurrence should notify the principal investigator (or another person in authority in absence of the principal investigator) immediately. The principal investigator should determine the immediate action to be taken. This may include requesting the support of the medical service to help identify the possibility of infection and disease. A thorough investigation by the medical service would include the collection and analysis of appropriate clinical specimens. For example, if self-inoculation, cuts or abrasions involving a potentially hazardous organism occurred, it would be advisable to collect serum samples immediately after the incident and at an appropriate time following the incident for the purpose of demonstrating, if possible, seroconversion. After a massive aerosol exposure, it may be advisable to obtain nasal and skin cultures to confirm the exposure. Throat washings may be of value in confirming exposure following accidental ingestion. In the event that a laboratory worker develops diarrhea or other gastrointestinal symptoms following an overt exposure, it may be advisable to obtain stool specimens for analysis of contamination with the research agent.

All major, unusual or extended illnesses of laboratory workers should be screened by the medical service for possible occupational origin

and recorded for future reference. Also, first-aid and lost-time occupational accidents, as well as accidents without personal injury but which result in exposure of the worker to a potential hazardous organism, should be noted in the worker's individual medical case file together with the result of examinations deemed appropriate by the physician at that time.

The investigation of accidents associated with recombinant DNA research should also include a review of techniques, procedures and types and uses of equipment that may have been involved in the accident. The investigation should also establish the circumstances leading to the accident. In addition, the investigation report should provide recommendations for preventing similar occurrences.

4. Medical Examinations

It is impossible to make specific recommendations concerning the need for either pre-assignment or periodic medical examinations for laboratory workers engaged in recombinant DNA research. Such recommendations must be determined on a case by case basis and will depend on the assessed hazards of the project and the individual needs of the laboratory worker. Where the potential for laboratory-acquired illness is known to exist, medical examinations are appropriate. Pre-assignment medical examinations, in this case, will establish base-line data that may provide the basis for comparison in the event a laboratory-acquired illness occurs.

If pre-assignment medical examinations are provided, they should include a medical history, physical examination, skin test for tuberculosis, serology, selected biochemical tests, a complete blood count, urinalysis, needed immunizations, vision testing and an audiometric examination. An electrocardiogram should be taken for persons over 40 years of age and a Papanicolau smear for women.

Periodic medical examinations of laboratory workers who are actively engaged in research with potentially hazardous organisms provide the opportunity to update the employee's work history and to ensure that the employee has the opportunity to bring to the attention of the medical service any condition which may require more extensive examination. Although scheduled periodic medical examinations would be the ideal, the realities of cost and the availability of medical manpower make individualized non-routine medical attention more rewarding to the laboratory worker and the institution alike. Updating the work and medical histories of the laboratory worker could be achieved by having the worker periodically prepare and transmit to the medical service an interval medical report. The medical service could review each report and determine whether medical consultation is required. Then an appointment could be scheduled with the laboratory worker, if necessary.

The following questions and requests for information would be recommended for inclusion in an interval medical report:

- Have you had any serious or extended illnesses since your last medical report?
- Have you been off work and/or hospitalized due to illness since your last medical examination or interval medical report.
- List the infectious agents you have been working with since your last medical examination or interval medical report.
- List the source(s) of DNA, the nature of inserted DNA sequences and the hosts and vectors you have used in recombinant DNA studies since your last medical examination or interval medical report.
- List all drugs or medications you use. Include prescriptions and over-the-counter medicines taken on a regular basis. Please state the name of each drug and the frequency with which it is taken. Be sure to note antibiotics and antacids.
- List any drugs or medication you take only occasionally (e.g., laxatives, pain medication).
- List any accidents that resulted in inoculations, ingestion or probable inhalation to recombinant DNA materials since your last medical examination or interval medical report.

Where a laboratory worker is maintained under an active medical surveillance program, it is recommended that a final medical examination be provided prior to termination of employment.

5. Medical Evaluation

Certain medical conditions may place a laboratory worker at increased risk. For example, laboratory workers who are undergoing treatment with steroids, immunosuppressive drugs or antibiotics, or are suffering from colitis, ileitis, active chronic diarrhea, or other gastrointestinal disorders, should have a medical evaluation to determine whether they should be engaged in research with potentially hazardous organisms during the time of their treatment or illness. Also pregnant women should be counseled as to the advisability of working in areas where the potential for exposure to potentially hazardous organisms is present. In order to ensure that appropriate guidance is provided, any changes in the health status of a laboratory worker, who is engaged in research with potentially hazardous organisms, should be brought to the attention of the medical service.

6. Records

The institution should maintain records of all laboratory workers who are involved in recombinant DNA research. As a minimum, these records should include a listing of the source DNA, vectors and host organisms used in recombinant DNA research and copies of all investigation reports concerning accidental exposures and serious or extended illnesses. These records should be included in the laboratory worker's medical file when the laboratory worker participates in an active medical surveillance program. Following termination of the laboratory worker's employment at the institution, the medical records should be maintained by a responsible medical authority for an appropriate period of time.

E. Training Aids, Materials and Courses

1. Slide-Tape Cassettes

- Introduction to Biohazards Control: Stock No. 176.54 (\$56.00).
- Research Laboratory Safety: Stock No. 176.79 (\$90.00).
- Assessment of Risk in the Cancer Virus Laboratory: NAC No. 009770 (\$12.50).
- Hazard Control in the Animal Laboratory: NAC No. 009432 (\$12.50).
- Selection of a Biological Safety Cabinet: NAC No. 000709 (\$19.00).
- Effective Use of the Laminar Flow Biological Safety Cabinet: NAC No. 005133 (\$12.50).
- Certification of Class II (Laminar Flow) Biological Safety Cabinets: NAC No. 009771 (\$17.25).
- Formaldehyde Decontamination of the Laminar Flow Biological Safety Cabinet: NAC No. 005137 (\$12.50).
- Basic Principles of Contamination Control (in preparation), National Audiovisual Center.

Note:

1. The first two items may be ordered from the National Safety Council, 444 North Michigan Avenue, Chicago, Illinois 60611.
2. The remaining items may be ordered prepaid, with check or money order payable to the National Archives Trust Fund mailed to: Sales Branch, National Audiovisual Center (GSA), Washington, D. C. 20409.

2. Films

- Laboratory Design for Microbiological Safety (M-1090).
- World Within a World (M-3766-X).
- Infectious Hazards of Bacteriological Techniques (M-382).
- Controlling Infectious Aerosols: Part 1—Precautions in Microbiology (T-3621-X).
- Controlling Infectious Aerosols: Part 2—Minimizing Equipment-Related Hazards (T-3622-X).
- Air Sampling for Microbiological Particles (M-926).
- Surface Sampling for Microorganisms (Rodac Method) (M-924).
- Surface Sampling for Microorganisms (Swab Method) (M-925).
- Plastic Isolators: New Tools for Medical Research (M-599).
- Safe Handling of Laboratory Animals (M-455).
- Handling the Laboratory Mouse (T-2617-X).
- Handling the Laboratory Guinea Pig (T-2618-X).

Note: The above films are available on loan without charge from:
Media Resources Branch, National Audiovisual Center (Annex),
Station K, Atlanta, Georgia 30324.

3. Training Manuals for Animal Caretakers

- American Association for Laboratory Animal Science, 1967. Manual for Laboratory Animal Technicians, Publication 67-3, Box 10, Joliet, Illinois 60434.
- American Association for Laboratory Animal Science, 1972. Syllabus for the Laboratory Animal Technologist, Publication 72-2, 2317 West Jefferson Street, Suite 208, Joliet, Illinois 60434.
- Ralston-Purina Company, 1961. Copyright (updated annually). Manual for Laboratory Animal Care, Checkerboard Square, St. Louis, Missouri 63199.

4. Training Courses

- Laboratory Safety Management

Sponsored or presented by the Center for Disease Control, Laboratory and Training Division, Bureau of Laboratories, Atlanta, Georgia 30333.

- Safety in the Laboratory

Sponsored or presented by the National Institute of Occupational Safety, Division of Training and Manpower Development, Robert A. Taft Laboratories, 4676 Columbia Parkway, Cincinnati, Ohio 45226.

- Biohazard and Injury Control in the Biomedical Laboratory.

- Biohazard Containment and Control for Recombinant DNA Molecules.

Note:

1. The last two courses are sponsored or presented by the National Cancer Institute, Office of Research Safety, NIH, Building 13, Room 2E45, Bethesda, Maryland 20014.
2. Information on dates and locations of courses can be obtained by writing to the sponsoring agency listed.

F. Reference Bibliography on Biological Safety

Published literature on safety in the microbiological laboratory is extensive. This bibliography has been prepared to cite but a few of the important articles in this field. The citations have been organized under 11 topic areas for easy reference. These articles should prove useful to investigators, safety professionals, and institutional officials who wish to acquire a further understanding of the principles of safety in the microbiological laboratory.

1. Biological Safety Guides, Manuals, and Standards

Lenette, E.H., et al. 1974. Laboratory Safety Regulations, Viral and Rickettsial Disease Laboratory. California State Department of Health, Berkeley, CA.

Medical Research Council. 1977. Guidelines for Handling Recombinant DNA Molecules and Animal Viruses and Cells. Minister of Supply and Services Canada, Cat. No.: MR21-1/1977 ISBN 0-662-00587-2.

National Cancer Institute. 1975. Safety Standards for Research Involving Chemical Carcinogens. DHEW Publication No. (NIH) 76-900. NIH, NCI, Bethesda, MD.

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In 1974, the National Cancer Institute issued Safety Standards for Research Involving Viruses. Control practices for moderate risk oncogenic viruses are included here to provide additional guidance to investigators who may use these viruses in recombinant DNA research. All known oncogenic viruses not presently classified as moderate risk oncogenic viruses may be safely handled by employing physical containment practices that are consistent with P2 requirements.

A. Moderate Risk Oncogenic Viruses

1. RNA Tumor Viruses: Feline Leukemia
Feline Sarcoma
Woolly Monkey Fibrosarcoma
Gibbon Ape Lymphosarcoma
2. DNA Tumor Viruses: Herpesvirus saimiri
Herpesvirus ateles
Yaba Pox Virus
Epstein-Barr Virus

B. Medical Surveillance

1. Preassignment Examinations

An appropriate preassignment physical examination shall be provided each person planning to work with moderate risk oncogenic viruses. The purpose of this examination is to establish a baseline against which changes can be measured and to determine whether there are any medical conditions that may lead to increased risk.

2. Periodic Examinations

Persons working with moderate risk oncogenic viruses shall be provided periodic physical examinations. The frequency shall be dependent upon the work circumstances and the age and sex of the employee.

3. Medical Evaluation

Persons with reduced immunologic competency, pregnant women, and patients under treatment with steroids or cytotoxic drugs shall receive a medical evaluation before work in areas where moderate risk oncogenic viruses are used.

4. Serum Collection

Serum shall be collected at the time of the preemployment physical examination to establish a baseline reference and on a semiannual basis thereafter in order to monitor serological changes. Serum also shall be collected prior to termination of employment and immediately after an accidental exposure and at an appropriate interval thereafter for serological testing.

C. Personnel Practices

1. Protective Clothing

a. Gloves shall be worn when using oncogenic viruses. Clean laboratory clothing shall be provided at least weekly. Protective clothing contaminated by oncogenic viruses shall be autoclaved.

b. Animal handlers shall use a complete clothing change, including pants and shirts or jumpsuits, safety-toed shoes or boots, head covers, and gloves. Clean clothing shall be provided at least weekly. Clothing contaminated by oncogenic viruses shall be autoclaved.

2. Protective Equipment

Personnel engaged in animal procedures where exposure to airborne particulates contaminated with oncogenic viruses could occur shall wear an appropriate half-face, filter-type mask. The mask shall not be worn outside the work area.

3. Eating, Drinking, and Smoking

There shall be no eating, drinking, smoking, chewing of gum or tobacco, application of cosmetics or storage of food in laboratories where oncogenic viruses are used.

4. Pipetting

Mechanical pipetting aids shall be used for all pipetting procedures.

D. Operational Practices

1. Access Control

a. The universal Biohazard symbol shall be prominently displayed at access points to all work areas where moderate risk viruses are used.

b. Only persons authorized by the laboratory supervisor shall enter work areas displaying the Biohazard symbol.

2. Identification and Storage of Materials

a. Storage vessels containing oncogenic viruses shall be labeled to provide identification of their contents.

b. An inventory of all oncogenic viruses shall be maintained.

3. Laboratory Transport

a. Nonbreakable impermeable closed containers shall be used during transport of oncogenic viruses through a building corridor or between buildings.

b. Contaminated materials that are transferred from work sites to decontamination and disposal staging areas shall be transported in a manner that prevents accidental spills.

4. Housekeeping

Housekeeping procedures that suppress the formation of aerosols such as the use of a wet mop or a vacuum cleaner equipped with a high-efficiency particulate air (HEPA) filter on the exhaust shall be used.

Dry sweeping and dry mopping shall be kept to a minimum.

5. Decontamination and Disposal

a. Contaminated glassware and similar materials shall be decontaminated before washing or disposal.

b. Liquid wastes containing oncogenic viruses shall be decontaminated, either chemically or by heat, before being discharged to the community sanitary sewer system.

c. Other contaminated wastes and animal carcasses shall be collected in impermeable containers that are closed prior to removal from the work area. Disposal shall be by incineration or other appropriate methods.

6. Packaging and Shipping

The packaging and shipping methods established by the Department of Health, Education, and Welfare for the transportation of etiologic agents [42CFR 72.25(c), 1972] shall be followed for all moderate risk viruses.

7. Vacuum Lines

Each vacuum service shall be protected with a disposable HEPA filter and a liquid trap. The effluent from aspiration of liquid containing oncogenic viruses should be collected in liquid traps containing concentrated disinfectant.

E. Biological Safety Cabinets

1. Use of Biological Safety Cabinets

a. Aerosol-generating processes such as blending, grinding, and sonicating shall be contained in biological safety cabinets.

b. Cell culture procedures involving moderate risk viruses shall be contained in biological safety cabinets.

c. Inoculated animals shall be kept in ventilated, ultraviolet, or filter top cages.

2. Performance Requirements

- a. Ventilated safety cabinets shall be tested annually.
- b. The average velocity of the airflow through the work opening of the cabinet shall be a minimum of 75 feet per minute.
- c. The exhaust air from ventilated safety cabinets shall be filtered with HEPA filters.

F. Facilities

Directional airflow shall be provided in laboratory areas and animal rooms where moderate risk oncogenic viruses are used. Air shall flow in the direction from areas of least potential contamination toward areas of greatest potential contamination. No recirculation of exhaust air from these areas shall be permitted without appropriate treatment.

KANSAS CITY UNIVERSITY
OF MEDICINE AND BIOSCIENCES
MAIN CAMPUS

BLOODBORNE PATHOGEN
EXPOSURE CONTROL PLAN

Revised
October, 2011

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SECTION I
PURPOSE OF THE PLAN

PURPOSE OF THE PLAN

The purpose of the Bloodborne Pathogen Exposure Control Plan (hereafter Exposure Control Plan) for Kansas City University of Medicine and Biosciences (KCUMB) is to promote safe work practices in an effort to **minimize** the incidence of illness and injury experienced by employees. Employees of KCUMB Physician Associates should refer to that location's Exposure Control Plan.

The Occupational Safety and Health Administration (OSHA) has enacted the Bloodborne Pathogen Standard with the purpose of "reducing occupational exposure to Hepatitis B Virus (HBV), Human Immunodeficiency Virus (HIV) and other bloodborne pathogens" that employees may encounter in their workplace.

KCUMB believes that there are a number of "good general principles" that should be followed when working with blood -borne pathogens, including:

- It is prudent to **minimize** all exposures to blood-borne pathogens.
- All blood and body fluids should be treated as potentially infectious.
- Our facility should institute as many engineering and work practice controls as possible to eliminate or minimize employee exposure to blood-borne pathogens.

We have implemented this Exposure Control Plan to meet the letter and intent of the OSHA Blood borne Pathogens Standard. The object of this plan is:

- To protect our employees from the health hazards associated with blood-borne pathogens.
- To provide appropriate treatment and counseling should an employee be exposed to blood-borne pathogens.

SECTION II
GENERAL PROGRAM MANAGEMENT

GENERAL PROGRAM MANAGEMENT

A. PROGRAM MANAGERS

Exposure Control Officer -
Chair of Biological Safety Committee

B. RESPONSIBLE PERSONS

There are three major "Categories of Responsibility" that are central to the effective implementation of our Exposure Control Plan. These are:

- The "Exposure Control Officer" working with the Occupational Health Officer and the Training Coordinator
- Department Chairs, Directors and Supervisors
- Our Employees

The following sections define the roles played by each of these groups in carrying out our plan.

EXPOSURE CONTROL OFFICER

The "Exposure Control Officer" will be responsible for overall management and support of our facility's Blood-borne Pathogens Compliance Program. Activities which are delegated to the Exposure Control Officer include, but are not limited to:

- Overall responsibility for implementing the Exposure Control Plan for the entire facility.
- Working with physicians and other employees to develop and administer any additional blood-borne pathogens policies and practices needed to support the effective implementation of this plan.
- Looking for ways to improve the Exposure Control Plan, as well as to revise and update the plan as necessary.
- Knowing current legal requirements concerning blood-borne pathogens.
- Acting as facility liaison during OSHA inspections.

- Coordinate training to all employees who have the potential for exposure to blood-borne pathogens.
- Assist facility management in keeping an ongoing updated record of personnel requiring training.
- Maintains appropriate training documentation such as "sign-in sheets".
- Annually reviews the training program with department chairs and directors to include appropriate new information.

The Chair of the Biological Safety Committee is the facility's Exposure Control Officer.

To assist the Exposure Control Officer in carrying out these duties, KCUMB has created the following positions:

- Occupational Health Officer (see page 26)
- OSHA required blood borne pathogens training coordinator (see page 30)

DEPARTMENT CHAIRS AND DIRECTORS

Department Chairs and Directors are responsible for exposure control in their respective areas. They work directly with the Exposure Control Officer and our employees to ensure that proper exposure control procedures are followed. For instance, Department Chairs and Directors are responsible for the collection, handling of and disposal of biohazardous waste generated in their areas, as well as receiving reports of exposure incidents of employees within their department.

EMPLOYEES

Our employees have the most important role in our Blood borne Pathogens Compliance Program, for the ultimate execution of much of our Exposure Control Plan rests in their hands. In this role they must:

- Know what tasks they perform that have occupational exposure.
- Attend the blood borne pathogen training sessions.
- Plan and conduct all operations in accordance with our work practice controls.
- Develop good personal hygiene habits.

C. AVAILABILITY OF EXPOSURE CONTROL PLAN TO EMPLOYEES

To help them with their efforts, our facility Exposure Control Plan is available to our employees at any time. Employees are advised of this availability during their education/training sessions. Copies of the Exposure Control Plan are kept in the office of the Chair of the Safety Committee and in research laboratories.

D. REVIEW AND UPDATE OF THE PLAN

- To ensure that our Exposure Control Plan is up-to-date, the plan will be reviewed and updated under the following circumstances:
- Annually, on or before the end of October of each year.
- Whenever new or modified tasks and procedures are implemented which affect occupational exposure of our employees.
- Whenever our employees' jobs are revised such that the new instances of occupational exposure may occur.
- Whenever we establish new functional positions within our facility that may involve exposure to blood-borne pathogens.

SECTION III
EXPOSURE DETERMINATION

EXPOSURE DETERMINATION

One of the keys to implementing a successful Exposure Control Plan is to identify those employees who may be at risk for exposure to blood borne pathogens, including HIV and HBV, hazardous materials and waste, infectious waste.

The attached list contains job classifications of those personnel who may have occupational exposure to human blood and/or other potentially infectious materials, possibly resulting in an exposure to blood-borne pathogens. The Exposure Control Officer will work with department chairs and directors to revise and update this list as classifications change.

Blood-borne Pathogens means pathogenic microorganisms that are present in human blood and can cause disease in humans. These pathogens include, but are not limited to, hepatitis B virus (HBV) and human immunodeficiency virus (HIV).

Hazardous materials and waste - materials, of which the handling, use, and storage are guided by local, state, or federal regulation (for example, OSHA's Regulations for Blood-borne Pathogens regarding the disposal of blood and blood-soaked items; Nuclear Regulatory Commission's regulations for the handling and disposal of radioactive waste) and hazardous energy sources (for example, ionizing or non-ionizing radiation, lasers, microwave, or ultrasound).

Regulated Waste means liquid or semi-liquid blood or other potentially infectious materials; contaminated items that would release blood or other potentially infectious materials in a liquid or semi-liquid state if compressed; items that are caked with dried blood or other potentially infectious materials and are capable of releasing these materials during handling; contaminated sharps; and pathological and microbiological wastes containing blood or other potentially infectious materials.

Reference: Federal Register

JOB CATEGORIES	TYPE	CATEGORY
Executive Administrative Managerial	HAZ,HIV & HBV,INF	III, III, III
Clinical Research Coordinator	HAZ,HIV & HBV,INF	I, I, I
Other Professionals	HAZ,HIV & HBV,INF	III, III, III
Academic Assistant	HAZ,HIV & HBV,INF	II, II, II
Clinical Research Super	HAZ,HIV & HBV,INF	I, I, I
Dir of Clinical Research	HAZ,HIV & HBV,INF	I, I, I
Research Assistant	HAZ,HIV & HBV,INF	I, I, I
Score 1 Coordinator	HAZ,HIV & HBV,INF	II, II, II
Score 1 RN	HAZ,HIV & HBV,INF	II, II, II
Technical & Paraprofessionals	HAZ,HIV & HBV,INF	III, III, III
Clerical & Secretarial	HAZ,HIV & HBV,INF	III, III, III
Asst. Coordinator Score 1	HAZ,HIV & HBV,INF	II, II, II
Clinical Res Cood II	HAZ,HIV & HBV,INF	II, II, II
OPP Fellowship	HAZ,HIV & HBV,INF	I, I, I
Research Asst. I	HAZ,HIV & HBV,INF	I, I, I
Student Asst I and II	HAZ,HIV & HBV,INF	II, II, II
Student Asst IV	HAZ,HIV & HBV,INF	I, I, I
Skilled Crafts	HAZ,HIV & HBV,INF	III, III, III
Service/ Maintenance	HAZ,HIV & HBV,INF	III, III, III
Custodian I and II	HAZ,HIV & HBV,INF	I, I, I
Security Officer	HAZ,HIV & HBV,INF	II, II, II
Faculty	HAZ,HIV & HBV,INF	II, II, II
Assoc. Prof Clinical Science	HAZ,HIV & HBV,INF	I, I, I
Assoc. Prof OB GYN	HAZ,HIV & HBV,INF	I, I, I
Assoc. Prof Surgery	HAZ,HIV & HBV,INF	I, I, I
Asst. Dean Clinical Science	HAZ,HIV & HBV,INF	I, I, I
Asst Prof Clinical Science	HAZ,HIV & HBV,INF	I, I, I
Asst Prof OB GYN	HAZ,HIV & HBV,INF	I, I, I
Asst. Prof Surgery	HAZ,HIV & HBV,INF	I, I, I
Prof Surgery	HAZ,HIV & HBV,INF	I, I, I
Prof BS Research	HAZ,HIV & HBV,INF	II, II, II
Prof Clinical Science	HAZ,HIV & HBV,INF	I, I, I
Prof OB GYN	HAZ,HIV & HBV,INF	I, I, I
Research Epidemiologist	HAZ,HIV & HBV,INF	III, III, III
Teaching Assistant	HAZ,HIV & HBV,INF	I, I, I
Instruction/Research Assistants	HAZ,HIV & HBV,INF	I, I, I

Type: HAZ = Hazardous Materials; HIV&HBV = Human Immunodeficiency Virus & Hepatitis B Virus; INF = Infectious Waste

Legend: Category I = High exposure to hazardous materials and infectious waste
Category II = Potential exposure to hazardous materials and infectious waste
Category III = Little or no exposure to hazardous materials and infectious waste

SECTION IV
METHODS OF COMPLIANCE

METHODS OF COMPLIANCE

We understand that there are a number of areas that must be addressed in order to effectively eliminate or minimize exposure to blood-borne pathogens in our facility. The first five areas we deal with in our plan are:

- The use of Standard Precautions - (formerly known as Universal Precautions),
- Establishing appropriate Engineering Controls,
- Implementing appropriate Work Practice Controls,
- Using necessary Personal Protective Equipment, and
- Implementing appropriate Housekeeping Procedures.

Each of these areas is reviewed with our employees during their blood borne pathogens related training (see the "Information and Training" section of this plan for additional information). By following the requirements of OSHA's Blood borne Pathogens Standard in these five areas, we feel that we will eliminate or minimize our employee's occupational exposure to blood borne pathogens as much as possible.

A. STANDARD PRECAUTIONS (formerly known as Universal Precautions)

In our facility we have observed the practice of "Standard Precautions" to prevent contact with blood and other potentially infectious materials. As a result, we treat all human blood and the following body fluids as if they are known to be infectious for HBV, HIV, and other blood borne pathogens:

- Semen
- Cerebrospinal fluid
- Pleural fluid
- Peritoneal fluid
- Saliva
- Vaginal secretions
- Synovial fluid
- Pericardial fluid
- Amniotic fluid

In circumstances where it is difficult or impossible to differentiate between body fluid types, we assume all body fluids to be potentially infectious.

Department Chairs and Directors are responsible to oversee our Standard Precautions Program.

B. ENGINEERING CONTROLS

One of the aspects of our Exposure Control Plan is the use of Engineering Controls to eliminate or minimize employee exposures to blood-borne pathogens. As a result, our facility employs equipment such as sharps disposal containers, self-sheathed IV piggyback needles and syringes, and ventilating laboratory hoods as appropriate.

Department Chairs and Directors periodically work with employees to review tasks and procedures performed in our facility where engineering controls can be implemented or updated.

In addition, the following engineering controls are used throughout our facility:

- Handwashing facilities (or antiseptic hand cleaners and towels), which are readily accessible to all employees who have the potential for exposure.
- Containers for contaminated sharps having the following characteristics:
 - Puncture-resistant
 - Labeled with a biohazard warning label
 - Leakproof
- Specimen containers which are:
 - Leakproof
 - Labeled with a biohazard warning label
 - Puncture resistant, if necessary
- Secondary containers which are:
 - Leakproof
 - Labeled with a biohazard warning label
 - Puncture resistant
- Eye wash stations
- B-D Safety-Lok Syringes

C. WORK PRACTICE CONTROLS

In addition to engineering controls, our facility uses a number of Work Practice Controls to help eliminate or minimize employee exposure to blood-borne pathogens.

Many of these Work Practice Controls have been in effect for some time.

The individuals in our facility who are responsible for overseeing the implementation of these Work Practice Controls are Department Chairs and Directors. They will work with supervisors and employees to implement Standard Precautions.

Our facility has adopted the following Work Practice Controls as part of our Blood-borne Pathogens Compliance Program:

- Employees wash their hands immediately, or as soon as feasible, after removal of gloves or other personal protective equipment.
- Following any contact of body areas with blood or any other infectious materials, employees wash their hands and any other exposed skin with soap and water as soon as possible. They also flush exposed mucous membranes with water. See Section V of this policy.
- Contaminated needles and other contaminated sharps are not bent, recapped, or removed unless:
 - It can be demonstrated that there is no feasible alternative.
 - The action is required by specific medical procedure.
 - In the two situations above, the recapping or needle removal is accomplished through the use of a medical device or a one-handed technique.
- Contaminated reusable sharps are placed in appropriate containers immediately, or as soon as possible, after use.
- Eating, drinking, applying cosmetics or lip balm, and handling contact lenses is prohibited in work areas where there is potential for exposure to blood-borne pathogens.
- Food and drink is not kept in refrigerators, freezers, on countertops or in other storage areas where blood or other potentially infectious materials are present.
- Mouth pipetting/suctioning of blood or other infectious materials is prohibited.

- All procedures involving blood or other infectious materials minimize splashing, spraying, or other actions generating droplets of these materials.
- Specimens of blood or other materials are placed in designated leakproof containers appropriately labeled for handling and storage.
- If outside contamination of a primary specimen container occurs, that container is placed within a second leakproof container and appropriately labeled, for handling and storage. (If the specimen can puncture the primary container, the secondary container must be puncture-resistant as well.)
- Equipment which becomes contaminated, is examined prior to servicing or shipping, and decontaminated as necessary (unless it can be demonstrated that decontamination is not feasible.)
 - An appropriate biohazard warning label is attached to any contaminated equipment, identifying the contaminated portion.
 - Information regarding the remaining contamination is conveyed to all affected employees, the equipment manufacturer and the equipment service representative prior to handling, servicing, or shipping.
- When a new employee comes to our facility, or an employee changes jobs within our facility, the following process takes place to ensure that they are trained in the appropriate work practice controls:
 - The employees job classification, tasks and procedures that he/she will perform are checked against the Job Classification list which we have identified in our Exposure Control Plan as those in which occupational exposure occurs.
 - If the employee is transferring from one job to another within the facility, the job classification, tasks, and procedures pertaining to their previous position are also checked against this list.
 - Based on this "cross-checking" the new job classification and/or tasks and procedures which will bring the employee into occupational exposure situations are identified.
 - The employee is then trained by their supervisor regarding any work practice controls that the employee is not experienced with.

D. PERSONAL PROTECTIVE EQUIPMENT

Personal Protective Equipment is our employee's last line of defense against blood borne pathogens. Because of this, our facility provides (at no cost to our employees) the Personal Protective Equipment that they need to protect themselves against such exposure. This equipment includes, but is not limited to:

- Gloves (NOTE: Hypoallergenic gloves are available to employees who are allergic to the gloves our facility normally uses).
- Gowns
- Laboratory coats
- Faceshields/masks
- Safety glasses/goggles
- Resuscitation bags
- Shoe covers
- Hoods/caps

The Department Chair or Director working with employees is responsible for ensuring that all departments and work areas have appropriate personal protective equipment available to employees.

Our employees are trained regarding the use of the appropriate personal protective equipment for their job classifications. Additional training is provided, when necessary, if an employee takes a new position or new job functions are added to their current position.

To determine whether additional training is needed, the employee's previous job classification and tasks are compared to those for any new job of function that they undertake. Any needed training is provided by their department manager or supervisor.

To ensure that personal protective equipment is not contaminated and is in the appropriate condition to protect employees from potential exposure, our facility adheres to the following practices:

- All personal protective equipment is inspected periodically and repaired or replaced as needed to maintain effectiveness.
- Reusable personal protective equipment is cleaned, laundered, and decontaminated as needed.

- Single-use personal protective equipment (or equipment that cannot, for whatever reason, be decontaminated) is disposed of in the infectious waste receptacle and forwarded to our facility's Environmental Services Department for proper disposal.

To make sure that this equipment is used as effectively as possible, our employees adhere to the following practices when using their personal protection equipment:

- Any garments penetrated by blood or other infectious materials are removed immediately, or as soon as feasible.
- All personal protective equipment is removed prior to leaving a work area.
- Gloves are worn in the following circumstances:
 - Whenever employees anticipate hand contact with potentially infectious materials.
 - When performing vascular access procedures.
 - When handling or touching contaminated items or surfaces.
- Disposable gloves are replaced as soon as practical after contamination or if they are torn, punctured, or otherwise lose their ability to function as an "exposure barrier".
- Utility gloves are decontaminated for reuse unless they are cracked, peeling, torn or exhibit other signs of deterioration, at which time they are disposed of.
- Masks and eye protection (such as goggles, face shields, etc.) are used whenever splashes or sprays may generate droplets of infectious materials.
- Protective clothing (such as a gown) is worn whenever potential exposure to the body is anticipated.
- Surgical caps and shoe covers are used in any instance where "gross contamination" is anticipated (such as autopsies and orthopedic surgery).

E. COMPLIANCE TO USE OF PERSONAL PROTECTIVE EQUIPMENT

To ensure employee compliance with the use of personal protective equipment, compliance will be monitored by supervisors and managers. Personnel who violate these regulations will be subject to disciplinary action.

F. HOUSEKEEPING

Maintaining our facility in a clean and sanitary condition is an important part of our Blood-borne Pathogens Compliance Program. To facilitate this, we have set up a written schedule for cleaning and decontamination of the various areas of the facility. The schedule can be found on the following pages and provides information on:

- The area to be cleaned/decontaminated.
- Time of scheduled work.
- Cleaners and disinfectants to be used.
- Any special instructions that are appropriate.

Using this schedule, our housekeeping/environmental services staff employs the following practices:

- All equipment and surfaces are cleaned and decontaminated after contact with blood or other potentially infectious materials:
 - Immediately (or as soon as feasible) when surfaces are overtly contaminated.
 - After any spill of blood or infectious materials.
- All pails, bins, cans and other receptacles intended for use routinely are inspected, cleaned, and decontaminated as soon as possible if visibly contaminated.
- Potentially contaminated broken glassware is picked up using mechanical means (such as dustpan and brush, tongs, forceps, etc.)

The Supervisor/Director of housekeeping is responsible for setting up our cleaning and decontamination schedule and making sure it is carried out within our facility.

We are also careful in our facility in handling regulated waste (including contaminated sharps, laundry, used bandages, and other potentially infectious materials).

- They are discarded and/or "bagged" in containers that are:
 - Closable
 - Puncture-resistant for sharps
 - Leakproof if the potential for fluid spill or leakage exists
 - Red in color and labeled with the appropriate biohazard warning label (if infectious waste).
 - Yellow in color and labeled with the appropriate biohazard warning label (if infectious linen).
 - Yellow in color and labeled with appropriate chemotherapy waste warning label (if chemotherapy waste).
- Containers for this regulated waste are located throughout our facility within easy access of our employees and as close as possible to the sources of the waste.
- Waste containers are maintained upright, routinely replaced, and not allowed to overfill.
- Contaminated laundry is handled as little as possible and is not sorted or rinsed where it is used.
- Whenever our employees move containers of regulated waste from one area to another, the containers are immediately closed and placed inside an appropriate secondary container (marked in the same manner as the primary container) if leakage is possible from the first container.
- If laundry is sent off site to a facility that does not utilize Standard Precautions, the laundry must be bagged and labeled as set forth above.

Department Chairs/Directors are responsible for the collection, handling and disposal of our facility's biohazardous waste generated in their areas.

Building Services Cleaning Schedule

Area	Frequency of Cleaning	Scheduled Cleaning
Public Areas	2 times	Daily
Offices		Daily
Restrooms	3 times	Daily
Classrooms		Daily
Kitchen & Cafeteria	2 times	Daily
Teaching Labs		Daily
Laboratories		Daily
Break Areas	2 times	Daily
Auditorium		Daily
Library Areas		Daily
Student Activity areas	2 times	Daily

Cleaners/Disinfectants Used

Product Name	Description	Instructions for Use
Quat 44	Cleaner, disinfectant, deodorizer, fungicide, mildewstat mildewstat and virucide.	Wear gloves
Quat 256	Cleaner, disinfectant, deodorizer, fungicide, virucide	Wear gloves
Neutral Germicide Cleaner # 49	Disinfectant cleaner, mildewstat, fungicide, virucide, deodorizer. (DCS) For dilution control system	Wear gloves
Glass Advantage # 41	Glass Cleaner (DCS) For Dilution Control	Wear gloves
Ultra Spray Cleaner # 42	All Purpose Cleaner - Walls (DCS) Dilution Control System	Wear gloves
Neutral Cleaner Plus # 46	All Purpose Cleaner - Floors (DCS) Dilution Control System	Wear gloves
Hi-Power Degreaser	Degreaser and cleaner for Food Services areas	Wear gloves
Spray N Away All Purpose Cleaner	Ready-to-use spray cleaner that lifts soil from many washable surfaces. For cleaning grease, oils, waxes, heel marks, food stains, fingerprints, etc. & must apply with damp rag & wipe clean.	Ready to Use Spray cleaner
Gojo Antimicrobial Lotion Soap	Hand soap for Lab areas & patient areas	Ready to use/No special instructions
Hand Sanitizer	Hand sanitizer for lab areas, patient areas, Food Service areas, classrooms, etc.	Ready to use
Lotion Skin cleanser	Hand soap for all restrooms	Ready to use

Hair & Body shampoo	Hair and body shampoo for all shower stalls	Ready to use
Extra strength CSP Cleaner	Cleaner for removing of hard water encrustations, soap film in showers, water film in showers, restrooms and rust deposits, etc.	Ready to Use Spray cleaner

SECTION V

HEPATITIS B VACCINATION,
POST EXPOSURE EVALUATION
AND FOLLOW-UP

HEPATITIS B VACCINATION, POST-EXPOSURE

EVALUATION AND FOLLOW-UP

A. HEPATITIS B VACCINATION

Hepatitis B vaccine and all medical evaluations, procedures and lab tests associated with such vaccination are available, free of charge, to all employees who have occupational exposure to blood or other potentially infectious materials. A list of job classifications of persons likely to have such occupational exposure is outlined in the section of this plan entitled "Exposure Determination."

This vaccine is made available at the time of initial hiring and if the employee chooses not to take the vaccine at that point in time, it is available on request.

It is the option of the employee whether or not to receive the Hepatitis B vaccine. However, the employee must understand that his/her possible occupational exposure to blood or other potential infectious materials could result in acquiring of Hepatitis B virus (HBV) infection.

The vaccination program consists of a series of three inoculations over a six month period. As part of their blood-borne pathogens training, our employees have received information regarding Hepatitis vaccination, including its safety and effectiveness.

The Exposure Control Officer is responsible for setting up and operating our vaccination program.

Vaccinations are performed under the supervision of a licensed physician or other healthcare professional. Employees who have declined to take part in the program have signed a declination form to that effect. Employees wishing to be vaccinated can call the Exposure Control Officer to make an appointment.

Booster doses are not currently recommended. However, if routine boosters are recommended at a later date, such booster doses will be made available.

B. POST-EXPOSURE EVALUATION AND FOLLOW-UP

It is KCUMB's purpose to confidentially evaluate, prophylax/treat and immediately follow-up on all occupational exposures to blood and body fluids via needle sticks, other sharps injury, mucous membrane or parenteral contact with blood or other potentially infectious materials.

Personnel who have exposure to blood or body fluids of another person (patient or staff) via "sharps' injury, mucous membrane or percutaneous route, must follow these steps:

- Thoroughly wash wound or exposed area with soap and water. If splashed in the eyes, flush with copious amounts of water or saline.

This process will help to physically remove contaminants

- Go to their Doctor or the Emergency Room within 24 hours for evaluation and to obtain baseline blood draws.
- Notify Department Chair, Director or supervisor and Occupational Health Officer.
 - Supervisor can investigate the incident in a timely manner and evaluate immediate steps to prevent further incidents, where possible.
- Fill out "Employee Incident" form.
 - Absolutely necessary for Workers' Compensation coverage.
 - This information will be used to determine effective strategies for preventing future exposures.
- Contact the Department Chair, Director or supervisor for processing.
- Identify source patient when possible . Provide this information to the Department Chair, director or supervisor.
 - This will help the staff and physician perform an accurate risk assessment.
 - The Safety Committee along with Department Chair or Director will be responsible for reviewing any employee exposure that results in a need for a modification of tasks or procedures performed by that employee.

- Post-exposure evaluation will consist of:

- Documentation of the route(s) of exposure and the circumstances under which the exposure incident occurred.
- Identification and documentation of the source individual (when known).
- Serological status will be determined by the following:
 - The source individual's blood will be tested as soon as possible and after consent is obtained in order to determine HBV and HIV infectivity.
 - When the source individual is already known to be infected with HBV or HIV, testing for the source individual's known HBV and HIV status is not repeated.
 - Results of the source individual's testing will be made available to the exposed employee and the employee will be informed of applicable laws and regulations concerning disclosures of the identify and infectious status of the source individual.
 - The exposed employee's blood will be collected as soon as possible and tested after consent is obtained.
 - If the employee consents to baseline blood collection, but does not give consent at that time for HIV serological testing, the sample will be preserved for at least 90 days.
 - If, within 90 days of the exposure, the employee elects to have the baseline sample tested, such testing will be conducted.
 - Post-exposure prophylaxis, when medically indicated, as recommended by the U.S. Public Health Service will consist of
 - Counseling
 - Evaluation of reported illnesses
 - Treatment, if indicated should be started within 24 hours.

C. COUNSELING

Counseling will be provided prior to testing for HIV and/or HBV after obtaining consent from the employee and the source patient. Results of the source patient's testing will be made available to the exposed employee and appropriate treatment will be provided. The employee will be informed of applicable laws and regulations concerning disclosure of the identify and infectious nature of the source individual.

D. INFORMATION PROVIDED TO THE HEALTHCARE PROFESSIONAL

We will ensure that the healthcare professional responsible for the employee's Hepatitis B vaccination is provided a copy of the federal regulations related to the bloodborne pathogens standards located at 29 C.F.R. §1910.1030.

We will also ensure that the healthcare professional evaluating an employee after an exposure event is provided with:

- A copy of the federal regulations related to the bloodborne pathogens standards located at 29 C.F.R. §1910.1030;
- A description of the exposed employee's duties as they relate to the exposure incident;
- Documentation of the route(s) of exposure and circumstances under which exposure occurred;
- Results of the source individual's blood testing, if available; and
- All medical records relevant to the appropriate treatment of the employee that we are required to maintain, including vaccination status.

E. HEALTHCARE PROFESSIONALS WRITTEN OPINION

A written opinion from the healthcare professional evaluating the exposure will be provided to the exposed employee. The written opinion will contain the following information:

- Whether Hepatitis B vaccination and Hepatitis B Immune Globulin (Recombivax) is indicated for the employee.
- Whether the employee has received the Hepatitis B vaccination.
- Confirmation by the employee's signature that he/she has been informed of the results of the evaluation.

- Whether anti-viral agents for HIV are indicated.
- Confirmation that the employee has been told about any medical conditions resulting from the exposure incident which requires further evaluation or treatment.

F. MEDICAL RECORDKEEPING

To make sure that we have medical information available to the healthcare professional evaluating the exposure, our facility maintains comprehensive medical records on our employees. The Occupational Health Officer is responsible for setting up and maintaining these records, which includes, but are not limited to, the following information:

- Name, department, position of employee
- Copy of the employee's Hepatitis B. Vaccination status with dates of any vaccinations
- Copies of the results of the examination, medical testing and follow-up procedures which took place as a result of an employee's exposure to blood borne pathogens.

As with all information in these areas, we recognize that it is important to keep the information in these medical records confidential. We will not disclose or report this information to anyone without our employee's written consent (except as required by law). These medical records will be retained for the duration of employment plus 30 years.

SECTION VI
LABELS AND SIGNS

LABELS AND SIGNS

For our employees the most obvious warning of possible exposure to blood-borne pathogens are biohazard labels. Because of this, we have implemented a comprehensive biohazard warning labeling program in our facility using labels of the type shown below. Department Chairs and directors are responsible for assuring the appropriate placement of labels and signs.

The following items in our facility were labeled:

- Containers of regulated waste
- Refrigerators/freezers containing blood or other potentially infectious materials
- Sharps disposal containers
- Other containers used to store, transport, or ship blood and other infectious materials
- Laundry bags/containers
- Contaminated equipment

On labels affixed to contaminated equipment we have also indicated which portion of the equipment is contaminated.

We recognize that biohazard signs must be posted at entrances to HIV and HBV research laboratories and production facilities. However, the laboratory in our facility performs only waived clinical diagnostic work, which is not covered by these special signage requirements.

BIOHAZARD LABEL



SECTION VII
INFORMATION AND TRAINING

INFORMATION AND TRAINING

Having well informed and educated employees is extremely important when attempting to eliminate or minimize our employees exposure to blood-borne pathogens. Because of this, all employees who have the potential for exposure to blood-borne pathogens are put through a comprehensive training program and furnished with as much information as possible on this issue.

Employees will be retrained at least annually to keep their knowledge current. Additionally, all new employees, as well as employees changing jobs or job functions, will be given any additional training their new position requires at the time of their new job assignment. Training will be offered at no cost to the employee with the employee being compensated for their time.

The Training Coordinator is responsible for seeing that employees who have the potential for exposure to blood borne pathogens receive this training.

A. TRAINING TOPICS

The topics covered in our training program include, but are not limited to:

- The Blood-borne Pathogens Standard itself
- The epidemiology and symptoms of blood-borne diseases
- The modes of transmission of blood-borne pathogens
- Our facility's Exposure Control Plan (and where employees can obtain a COPY)
- A review of the use and limitations of methods that will prevent or reduce exposure, including:
 - Engineering Controls
 - Work Practice Controls
 - Personal Protective Equipment

- Selection and use of personal protective equipment including:
 - Types available
 - Proper use
 - Location within facility
 - Handling
 - Disposal
 - Removal
- Visual warnings of biohazard within our facility including labels and signs
- Information on the Hepatitis B Vaccine, including its:
 - Efficacy
 - Safety
 - Method of administration
 - Benefits of vaccination
 - Our facility's free vaccination program
- Actions to take and persons to contact in an emergency involving blood or other potentially infectious materials
- The procedure to follow if an exposure incident occurs, including incident reporting
- Information on the post-exposure evaluation and follow-up, including medical consultation, that our facility will provide.

B. TRAINING METHODS

Our facility's training presentations make use of several training techniques including, but not limited to:

- Classroom-type atmosphere with personal instruction
- Videotapes
- Training manual/employee handouts

Because we feel that employees need an opportunity to ask questions and interact with their instructors, time is specifically allotted for these activities in each training session.

C. RECORDKEEPING

To facilitate the training of our employees, as well as to document the training process, we maintain training records containing the following information:

- Dates of all training sessions
- Contents of the training sessions
- Names and qualifications of the instructors
- Names and job titles of employees attending the training sessions

These training records are available for examination and copying to our employees and their representatives, as well as OSHA and its representatives

SECTION VIII
DEFINITIONS

DEFINITIONS

Blood - Human blood, human blood components, and products made from human blood

Blood-borne Pathogens - pathogenic microorganisms that are present in human blood and can cause disease in humans. These pathogens include, but are not limited to, Hepatitis B virus (HBV) and human immunodeficiency virus (HIV).

Contaminated - presence or the reasonable anticipated presence of blood or other potentially infectious materials on an item or surface

Contaminated Laundry - laundry which has been soiled with blood or other potentially infectious materials or may contain sharps

Contaminated Sharps - any contaminated objective that can penetrate the skin including, but not limited to, needles, scalpels, broken glass, broken capillary tubes, and exposed ends of dental wires

Decontamination - use of physical or chemical means to remove, inactivate, or destroy blood-borne pathogens on a surface or item to the point where they are no longer capable of transmitting infectious particles and the surface or item is rendered safe for handling, use or disposal

Engineering Controls - controls (i.e., sharps disposal containers, self-sheathing needles) that isolate or remove the blood-borne pathogens hazard from the work place

Exposure Incident - specific eye, mouth, other mucous membrane, non-intact skin, or parenteral contact with blood or other potentially infectious materials that results from the performance of an employee's duties

HBV - Hepatitis B virus

HIV - human immunodeficiency virus

Licensed Healthcare Professional - person whose legally permitted scope of practice allows him/her to independently perform the activities required by the Hepatitis B vaccination and post-exposure evaluation and follow-up

Occupational Exposure - reasonable anticipated skin, eye, mucous membrane or parenteral contact with blood or other potentially infectious materials that may result from the performance of an employee's duties

Parenteral - piercing mucous membranes or the skin barrier through such events as needlesticks, human bites, cuts, and abrasions

Personal Protective Equipment - specialized clothing or equipment worn by an employee for protection against a hazard (NOTE: General work clothes not intended to function as protection against a hazard are not considered to be personal protective equipment).

Regulated Waste - liquid or semi-liquid blood or other potentially infectious materials; contaminated items that would release blood or other potentially infectious materials in a liquid or semi-liquid state if compressed; items that are caked with dried blood or other potentially infectious materials and are capable of releasing these materials during handling; contaminated sharps; and pathological and microbiological wastes containing blood or other potentially infectious materials

Source Individual - any individual, living or dead, whose blood or other potentially infectious materials may be a source of occupational exposure to the employee. Examples include, but are not limited to, hospital and clinic patients; clients in institutions for the developmentally disabled; trauma victims; clients of drug and alcohol treatment facilities; residents of hospices and nursing homes; human remains; and individuals who donate or sell blood or blood components

Sterilize - use of a physical or chemical procedure to destroy all microbial life including highly resistant bacterial endospores

Standard Precautions - an approach to infection control. According to this concept, all human blood and certain body fluids are treated as if known to be infectious for HBV, HIV, and other bloodborne pathogens

Work Practice Controls - controls that reduce the likelihood of exposure to altering the manner in which a task is performed (i.e., prohibiting recapping of needles by a two-handed technique)

SECTION IX
STANDARD PRECAUTIONS

SPECIFIC GUIDELINES FOR
KANSAS CITY UNIVERSITY OF
MEDICINE AND BIOSCIENCES

STANDARD PRECAUTIONS

General Information:

- Standard precautions are designed to protect employees from exposure to potentially infectious agents through the use of barriers such as gloves, gowns, masks, and protective eyewear.
- All patients have organisms present in their body substances such as respiratory secretions, feces, oral secretions, emesis, and sometimes urine and wounds, that are causing "colonization" if not "infection".
- Colonized body substances can be a major reservoir for multiple-drug-resistant organisms that can be transmitted from patient to patient on the hands of personnel.
- Standard precautions reduce the risks of such transmission by the consistent use of barriers whenever the employee is likely to contact any body substance.
- Contact not involving blood/body substances, or contact with items not contaminated with such, does not require the use of protective barriers.
- If unanticipated exposure occurs, wash hands immediately, follow KCUMB policy for exposure.
- If injury (puncture wound, needlestick, or mucous membrane exposure) occurs, report and follow KCUMB policy for exposure.
- These guidelines are the minimum requirements recommended during controlled situations, to protect the employee from potentially infectious agents. Judgment is required on the part of the employee to assess the need for additional barrier protection in less controlled situations.
- Other barriers may be required to protect the patient during certain procedures.
- If an employee has an open cut or abrasion on their hands, they are responsible for protecting it through the use of gloves.
- Sterile technique is to be used during sterile procedures.
- Handwashing remains the number one factor in preventing the spread of infection.

Precautionary measures in addition to handwashing after contact

- No additional precautions are necessary unless there is contact with patient's blood or body substances, at which time gloves should be worn.
- Other departments, not listed, that do not have contact with patient's blood or body substances do not require any special precautions.

APPENDIX

FEDERAL REGISTER

Department of Labor
Occupational Safety and Health Administration

29 CFR 1910.1030

Occupational Exposure to Bloodborne Pathogens
Final Rule

U.S. Department of Labor
Occupational Safety & Health Administration

29 CFR 1910.1030
Occupational Safety and Health Standards
Bloodborne Pathogens

[1910.1030\(a\)](#)

Scope and Application. This section applies to all occupational exposure to blood or other potentially infectious materials as defined by paragraph (b) of this section.

[1910.1030\(b\)](#)

Definitions. For purposes of this section, the following shall apply:

Assistant Secretary means the Assistant Secretary of Labor for Occupational Safety and Health, or designated representative.

Blood means human blood, human blood components, and products made from human blood.

Bloodborne Pathogens means pathogenic microorganisms that are present in human blood and can cause disease in humans. These pathogens include, but are not limited to, hepatitis B virus (HBV) and human immunodeficiency virus (HIV).

Clinical Laboratory means a workplace where diagnostic or other screening procedures are performed on blood or other potentially infectious materials.

Contaminated means the presence or the reasonably anticipated presence of blood or other potentially infectious materials on an item or surface.

Contaminated Laundry means laundry which has been soiled with blood or other potentially infectious materials or may contain sharps.

Contaminated Sharps means any contaminated object that can penetrate the skin including, but not limited to, needles, scalpels, broken glass, broken capillary tubes, and exposed ends of dental wires.

Decontamination means the use of physical or chemical means to remove, inactivate, or destroy bloodborne pathogens on a surface or item to the point where they are no longer capable of transmitting infectious particles and the surface or item is rendered safe for handling, use, or disposal.

Director means the Director of the National Institute for Occupational Safety and Health, U.S. Department of Health and Human Services, or designated representative.

Engineering Controls means controls (e.g., sharps disposal containers, self-sheathing needles, safer medical devices, such as sharps with engineered sharps injury protections and needleless systems) that isolate or remove the bloodborne pathogens hazard from the workplace.

Exposure Incident means a specific eye, mouth, other mucous membrane, non-intact skin, or

parenteral contact with blood or other potentially infectious materials that results from the performance of an employee's duties.

Handwashing Facilities means a facility providing an adequate supply of running potable water, soap, and single-use towels or air-drying machines.

Licensed Healthcare Professional is a person whose legally permitted scope of practice allows him or her to independently perform the activities required by paragraph (f) Hepatitis B Vaccination and Post-exposure Evaluation and Follow-up.

HBV means hepatitis B virus.

HIV means human immunodeficiency virus.

Needleless systems means a device that does not use needles for:

(1) The collection of bodily fluids or withdrawal of body fluids after initial venous or arterial access is established; (2) The administration of medication or fluids; or (3) Any other procedure involving the potential for occupational exposure to bloodborne pathogens due to percutaneous injuries from contaminated sharps.

Occupational Exposure means reasonably anticipated skin, eye, mucous membrane, or parenteral contact with blood or other potentially infectious materials that may result from the performance of an employee's duties.

Other Potentially Infectious Materials means (1) The following human body fluids: semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva in dental procedures, any body fluid that is visibly contaminated with blood, and all body fluids in situations where it is difficult or impossible to differentiate between body fluids; (2) Any unfixed tissue or organ (other than intact skin) from a human (living or dead); and (3) HIV-containing cell or tissue cultures, organ cultures, and HIV- or HBV-containing culture medium or other solutions; and blood, organs, or other tissues from experimental animals infected with HIV or HBV.

Parenteral means piercing mucous membranes or the skin barrier through such events as needlesticks, human bites, cuts, and abrasions.

Personal Protective Equipment is specialized clothing or equipment worn by an employee for protection against a hazard. General work clothes (e.g., uniforms, pants, shirts or blouses) not intended to function as protection against a hazard are not considered to be personal protective equipment.

Production Facility means a facility engaged in industrial-scale, large-volume or high concentration production of HIV or HBV.

Regulated Waste means liquid or semi-liquid blood or other potentially infectious materials; contaminated items that would release blood or other potentially infectious materials in a liquid or semi-liquid state if compressed; items that are caked with dried blood or other potentially infectious materials and are capable of releasing these materials during handling; contaminated sharps; and pathological and microbiological wastes containing blood or other potentially infectious materials.

Research Laboratory means a laboratory producing or using research-laboratory-scale amounts of HIV or HBV. Research laboratories may produce high concentrations of HIV or HBV but not in the volume found in production facilities.

Sharps with engineered sharps injury protections means a nonneedle sharp or a needle device used for withdrawing body fluids, accessing a vein or artery, or administering medications or other fluids, with a built-in safety feature or mechanism that effectively reduces the risk of an exposure incident.

Source Individual means any individual, living or dead, whose blood or other potentially infectious materials may be a source of occupational exposure to the employee. Examples include, but are not limited to, hospital and clinic patients; clients in institutions for the developmentally disabled; trauma victims; clients of drug and alcohol treatment facilities; residents of hospices and nursing homes; human remains; and individuals who donate or sell blood or blood components.

Sterilize means the use of a physical or chemical procedure to destroy all microbial life including highly resistant bacterial endospores.

Universal Precautions is an approach to infection control. According to the concept of Universal Precautions, all human blood and certain human body fluids are treated as if known to be infectious for HIV, HBV, and other bloodborne pathogens.

Work Practice Controls means controls that reduce the likelihood of exposure by altering the manner in which a task is performed (e.g., prohibiting recapping of needles by a two-handed technique).

1910.1030(c)

Exposure Control --

1910.1030(c)(1)

Exposure Control Plan.

1910.1030(c)(1)(i)

Each employer having an employee(s) with occupational exposure as defined by paragraph (b) of this section shall establish a written Exposure Control Plan designed to eliminate or minimize employee exposure.

1910.1030(c)(1)(ii)

The Exposure Control Plan shall contain at least the following elements:

1910.1030(c)(1)(ii)(A)

The exposure determination required by paragraph (c)(2),

1910.1030(c)(1)(ii)(B)

The schedule and method of implementation for paragraphs (d) Methods of Compliance, (e) HIV and HBV Research Laboratories and Production Facilities, (f) Hepatitis B Vaccination and Post-Exposure Evaluation and Follow-up, (g) Communication of Hazards to Employees, and (h) Recordkeeping, of this standard, and

1910.1030(c)(1)(ii)(C)

The procedure for the evaluation of circumstances surrounding exposure incidents as required by paragraph (f)(3)(i) of this standard.

1910.1030(c)(1)(iii)

Each employer shall ensure that a copy of the Exposure Control Plan is accessible to employees in accordance with 29 CFR 1910.1020(e).

1910.1030(c)(1)(iv)

The Exposure Control Plan shall be reviewed and updated at least annually and whenever necessary to reflect new or modified tasks and procedures which affect occupational exposure and to reflect new or revised employee positions with occupational exposure. The review and update of such plans shall also:

1910.1030(c)(1)(iv)(A)

Reflect changes in technology that eliminate or reduce exposure to bloodborne pathogens; and

1910.1030(c)(1)(iv)(B)

Document annually consideration and implementation of appropriate commercially available and effective safer medical devices designed to eliminate or minimize occupational exposure.

1910.1030(c)(1)(v)

An employer, who is required to establish an Exposure Control Plan shall solicit input from non-managerial employees responsible for direct patient care who are potentially exposed to injuries from contaminated sharps in the identification, evaluation, and selection of effective engineering and work practice controls and shall document the solicitation in the Exposure Control Plan.

1910.1030(c)(1)(vi)

The Exposure Control Plan shall be made available to the Assistant Secretary and the Director upon request for examination and copying.

1910.1030(c)(2)

Exposure Determination.

1910.1030(c)(2)(i)

Each employer who has an employee(s) with occupational exposure as defined by paragraph (b) of this section shall prepare an exposure determination. This exposure determination shall contain the following:

1910.1030(c)(2)(i)(A)

A list of all job classifications in which all employees in those job classifications have occupational exposure;

1910.1030(c)(2)(i)(B)

A list of job classifications in which some employees have occupational exposure, and

1910.1030(c)(2)(i)(C)

A list of all tasks and procedures or groups of closely related task and procedures in which occupational exposure occurs and that are performed by employees in job classifications listed in accordance with the provisions of paragraph (c)(2)(i)(B) of this standard.

1910.1030(c)(2)(ii)

This exposure determination shall be made without regard to the use of personal protective equipment.

1910.1030(d)

Methods of Compliance --

1910.1030(d)(1)

General. Universal precautions shall be observed to prevent contact with blood or other potentially infectious materials. Under circumstances in which differentiation between body fluid types is difficult or impossible, all body fluids shall be considered potentially infectious materials.

1910.1030(d)(2)

Engineering and Work Practice Controls.

1910.1030(d)(2)(i)

Engineering and work practice controls shall be used to eliminate or minimize employee exposure. Where occupational exposure remains after institution of these controls, personal protective equipment shall also be used.

1910.1030(d)(2)(ii)

Engineering controls shall be examined and maintained or replaced on a regular schedule to ensure their effectiveness.

1910.1030(d)(2)(iii)

Employers shall provide handwashing facilities which are readily accessible to employees.

1910.1030(d)(2)(iv)

When provision of handwashing facilities is not feasible, the employer shall provide either an appropriate antiseptic hand cleanser in conjunction with clean cloth/paper towels or antiseptic towelettes. When antiseptic hand cleansers or towelettes are used, hands shall be washed with soap and running water as soon as feasible.

1910.1030(d)(2)(v)

Employers shall ensure that employees wash their hands immediately or as soon as feasible after removal of gloves or other personal protective equipment.

1910.1030(d)(2)(vi)

Employers shall ensure that employees wash hands and any other skin with soap and water, or flush mucous membranes with water immediately or as soon as feasible following contact of such body areas with blood or other potentially infectious materials.

1910.1030(d)(2)(vii)

Contaminated needles and other contaminated sharps shall not be bent, recapped, or removed except as noted in paragraphs (d)(2)(vii)(A) and (d)(2)(vii)(B) below. Shearing or breaking of contaminated needles is prohibited.

1910.1030(d)(2)(vii)(A)

Contaminated needles and other contaminated sharps shall not be bent, recapped or removed unless the employer can demonstrate that no alternative is feasible or that such action is required by a specific medical or dental procedure.

1910.1030(d)(2)(vii)(B)

Such bending, recapping or needle removal must be accomplished through the use of a mechanical device or a one-handed technique.

1910.1030(d)(2)(viii)

Immediately or as soon as possible after use, contaminated reusable sharps shall be placed in appropriate containers until properly reprocessed. These containers shall be:

1910.1030(d)(2)(viii)(A)

Puncture resistant;

1910.1030(d)(2)(viii)(B)

Labeled or color-coded in accordance with this standard;

1910.1030(d)(2)(viii)(C)

Leakproof on the sides and bottom; and

1910.1030(d)(2)(viii)(D)

In accordance with the requirements set forth in paragraph (d)(4)(ii)(E) for reusable sharps.

1910.1030(d)(2)(ix)

Eating, drinking, smoking, applying cosmetics or lip balm, and handling contact lenses are prohibited in work areas where there is a reasonable likelihood of occupational exposure.

1910.1030(d)(2)(x)

Food and drink shall not be kept in refrigerators, freezers, shelves, cabinets or on countertops or benchtops where blood or other potentially infectious materials are present.

1910.1030(d)(2)(xi)

All procedures involving blood or other potentially infectious materials shall be performed in such a manner as to minimize splashing, spraying, spattering, and generation of droplets of these substances.

1910.1030(d)(2)(xii)

Mouth pipetting/suctioning of blood or other potentially infectious materials is prohibited.

1910.1030(d)(2)(xiii)

Specimens of blood or other potentially infectious materials shall be placed in a container which prevents leakage during collection, handling, processing, storage, transport, or shipping.

1910.1030(d)(2)(xiii)(A)

The container for storage, transport, or shipping shall be labeled or color-coded according to paragraph (g)(1)(i) and closed prior to being stored, transported, or shipped. When a facility utilizes Universal Precautions in the handling of all specimens, the labeling/color-coding of specimens is not necessary provided containers are recognizable as containing specimens. This exemption only applies while such specimens/containers remain within the facility. Labeling or color-coding in accordance with paragraph (g)(1)(i) is required when such specimens/containers leave the facility.

1910.1030(d)(2)(xiii)(B)

If outside contamination of the primary container occurs, the primary container shall be placed within a second container which prevents leakage during handling, processing, storage, transport, or shipping and is labeled or color-coded according to the requirements of this standard.

1910.1030(d)(2)(xiii)(C)

If the specimen could puncture the primary container, the primary container shall be placed within a secondary container which is puncture-resistant in addition to the above characteristics.

1910.1030(d)(2)(xiv)

Equipment which may become contaminated with blood or other potentially infectious materials shall be examined prior to servicing or shipping and shall be decontaminated as necessary, unless the employer can demonstrate that decontamination of such equipment or portions of such equipment is not feasible.

1910.1030(d)(2)(xiv)(A)

A readily observable label in accordance with paragraph (g)(1)(i)(H) shall be attached to the equipment stating which portions remain contaminated.

1910.1030(d)(2)(xiv)(B)

The employer shall ensure that this information is conveyed to all affected employees, the servicing representative, and/or the manufacturer, as appropriate, prior to handling, servicing, or shipping so that appropriate precautions will be taken.

1910.1030(d)(3)

Personal Protective Equipment --

1910.1030(d)(3)(i)

Provision. When there is occupational exposure, the employer shall provide, at no cost to the employee, appropriate personal protective equipment such as, but not limited to, gloves, gowns, laboratory coats, face shields or masks and eye protection, and mouthpieces, resuscitation bags, pocket masks, or other ventilation devices. Personal protective equipment will be considered "appropriate" only if it does not permit blood or other potentially infectious materials to pass through to or reach the employee's work clothes, street clothes, undergarments, skin, eyes, mouth, or other mucous membranes under normal conditions of use and for the duration of time which the protective equipment will be used.

1910.1030(d)(3)(ii)

Use. The employer shall ensure that the employee uses appropriate personal protective equipment unless the employer shows that the employee temporarily and briefly declined to use personal protective equipment when, under rare and extraordinary circumstances, it was the employee's professional judgment that in the specific instance its use would have prevented the delivery of health care or public safety services or would have posed an increased hazard to the safety of the worker or co-worker. When the employee makes this judgement, the circumstances shall be investigated and documented in order to determine whether changes can be instituted to prevent such occurrences in the future.

1910.1030(d)(3)(iii)

Accessibility. The employer shall ensure that appropriate personal protective equipment in the appropriate sizes is readily accessible at the worksite or is issued to employees. Hypoallergenic gloves, glove liners, powderless gloves, or other similar alternatives shall be readily accessible to those employees who are allergic to the gloves normally provided.

1910.1030(d)(3)(iv)

Cleaning, Laundering, and Disposal. The employer shall clean, launder, and dispose of personal protective equipment required by paragraphs (d) and (e) of this standard, at no cost to the employee.

1910.1030(d)(3)(v)

Repair and Replacement. The employer shall repair or replace personal protective equipment as needed to maintain its effectiveness, at no cost to the employee.

1910.1030(d)(3)(vi)

If a garment(s) is penetrated by blood or other potentially infectious materials, the garment(s) shall be removed immediately or as soon as feasible.

1910.1030(d)(3)(vii)

All personal protective equipment shall be removed prior to leaving the work area.

1910.1030(d)(3)(viii)

When personal protective equipment is removed it shall be placed in an appropriately designated area or container for storage, washing, decontamination or disposal.

1910.1030(d)(3)(ix)

Gloves. Gloves shall be worn when it can be reasonably anticipated that the employee may have hand contact with blood, other potentially infectious materials, mucous membranes, and non-intact skin; when performing vascular access procedures except as specified in paragraph (d)(3)(ix)(D); and when handling or touching contaminated items or surfaces.

1910.1030(d)(3)(ix)(A)

Disposable (single use) gloves such as surgical or examination gloves, shall be replaced as soon as practical when contaminated or as soon as feasible if they are torn, punctured, or when their ability to function as a barrier is compromised.

1910.1030(d)(3)(ix)(B)

Disposable (single use) gloves shall not be washed or decontaminated for re-use.

1910.1030(d)(3)(ix)(C)

Utility gloves may be decontaminated for re-use if the integrity of the glove is not compromised. However, they must be discarded if they are cracked, peeling, torn, punctured, or exhibit other signs of deterioration or when their ability to function as a barrier is compromised.

1910.1030(d)(3)(ix)(D)

If an employer in a volunteer blood donation center judges that routine gloving for all phlebotomies is not necessary then the employer shall:

1910.1030(d)(3)(ix)(D)(1)

Periodically reevaluate this policy;

1910.1030(d)(3)(ix)(D)(2)

Make gloves available to all employees who wish to use them for phlebotomy;

1910.1030(d)(3)(ix)(D)(3)

Not discourage the use of gloves for phlebotomy; and

1910.1030(d)(3)(ix)(D)(4)

Require that gloves be used for phlebotomy in the following circumstances:

1910.1030(d)(3)(ix)(D)(4)(i)

When the employee has cuts, scratches, or other breaks in his or her skin;

1910.1030(d)(3)(ix)(D)(4)(ii)

When the employee judges that hand contamination with blood may occur, for example, when performing phlebotomy on an uncooperative source individual; and

1910.1030(d)(3)(ix)(D)(4)(iii)

When the employee is receiving training in phlebotomy.

1910.1030(d)(3)(x)

Masks, Eye Protection, and Face Shields. Masks in combination with eye protection devices, such as goggles or glasses with solid side shields, or chin-length face shields, shall be worn whenever splashes, spray, spatter, or droplets of blood or other potentially infectious materials may be generated and eye, nose, or mouth contamination can be reasonably anticipated.

1910.1030(d)(3)(xi)

Gowns, Aprons, and Other Protective Body Clothing. Appropriate protective clothing such as, but not limited to, gowns, aprons, lab coats, clinic jackets, or similar outer garments shall be worn in occupational exposure situations. The type and characteristics will depend upon the task and degree of exposure anticipated.

1910.1030(d)(3)(xii)

Surgical caps or hoods and/or shoe covers or boots shall be worn in instances when gross contamination can reasonably be anticipated (e.g., autopsies, orthopaedic surgery).

1910.1030(d)(4)

Housekeeping --

1910.1030(d)(4)(i)

General. Employers shall ensure that the worksite is maintained in a clean and sanitary condition. The employer shall determine and implement an appropriate written schedule for cleaning and method of decontamination based upon the location within the facility, type of surface to be cleaned, type of soil present, and tasks or procedures being performed in the area.

1910.1030(d)(4)(ii)

All equipment and environmental and working surfaces shall be cleaned and decontaminated after contact with blood or other potentially infectious materials.

1910.1030(d)(4)(ii)(A)

Contaminated work surfaces shall be decontaminated with an appropriate disinfectant after completion of procedures; immediately or as soon as feasible when surfaces are overtly contaminated or after any spill of blood or other potentially infectious materials; and at the end of the work shift if the surface may have become contaminated since the last cleaning.

1910.1030(d)(4)(ii)(B)

Protective coverings, such as plastic wrap, aluminum foil, or imperviously-backed absorbent paper used to cover equipment and environmental surfaces, shall be removed and replaced as soon as feasible when they become overtly contaminated or at the end of the work shift if they may have become contaminated during the shift.

1910.1030(d)(4)(ii)(C)

All bins, pails, cans, and similar receptacles intended for reuse which have a reasonable likelihood for becoming contaminated with blood or other potentially infectious materials shall be inspected and decontaminated on a regularly scheduled basis and cleaned and decontaminated immediately or as soon as feasible upon visible contamination.

1910.1030(d)(4)(ii)(D)

Broken glassware which may be contaminated shall not be picked up directly with the hands. It shall be cleaned up using mechanical means, such as a brush and dust pan, tongs, or forceps.

1910.1030(d)(4)(ii)(E)

Reusable sharps that are contaminated with blood or other potentially infectious materials shall not be stored or processed in a manner that requires employees to reach by hand into the containers where these sharps have been placed.

1910.1030(d)(4)(iii)

Regulated Waste --

1910.1030(d)(4)(iii)(A)

Contaminated Sharps Discarding and Containment.

1910.1030(d)(4)(iii)(A)(1)

Contaminated sharps shall be discarded immediately or as soon as feasible in containers that are:

1910.1030(d)(4)(iii)(A)(1)(i)

Closable;

1910.1030(d)(4)(iii)(A)(1)(ii)

Puncture resistant;

1910.1030(d)(4)(iii)(A)(1)(iii)

Leakproof on sides and bottom; and

1910.1030(d)(4)(iii)(A)(1)(iv)

Labeled or color-coded in accordance with paragraph (g)(1)(i) of this standard.

1910.1030(d)(4)(iii)(A)(2)

During use, containers for contaminated sharps shall be:

1910.1030(d)(4)(iii)(A)(2)(i)

Easily accessible to personnel and located as close as is feasible to the immediate area where sharps are used or can be reasonably anticipated to be found (e.g., laundries);

1910.1030(d)(4)(iii)(A)(2)(ii)

Maintained upright throughout use; and

1910.1030(d)(4)(iii)(A)(2)(iii)

Replaced routinely and not be allowed to overfill.

1910.1030(d)(4)(iii)(A)(3)

When moving containers of contaminated sharps from the area of use, the containers shall be:

1910.1030(d)(4)(iii)(A)(3)(i)

Closed immediately prior to removal or replacement to prevent spillage or protrusion of contents during handling, storage, transport, or shipping;

1910.1030(d)(4)(iii)(A)(3)(ii)

Placed in a secondary container if leakage is possible. The second container shall be:

1910.1030(d)(4)(iii)(A)(3)(ii)(A)

Closable;

1910.1030(d)(4)(iii)(A)(3)(ii)(B)

Constructed to contain all contents and prevent leakage during handling, storage, transport, or shipping; and

1910.1030(d)(4)(iii)(A)(3)(ii)(C)

Labeled or color-coded according to paragraph (g)(1)(i) of this standard.

1910.1030(d)(4)(iii)(A)(4)

Reusable containers shall not be opened, emptied, or cleaned manually or in any other manner which would expose employees to the risk of percutaneous injury.

1910.1030(d)(4)(iii)(B)

Other Regulated Waste Containment --

1910.1030(d)(4)(iii)(B)(1)

Regulated waste shall be placed in containers which are:

1910.1030(d)(4)(iii)(B)(1)(i)

Closable;

1910.1030(d)(4)(iii)(B)(1)(ii)

Constructed to contain all contents and prevent leakage of fluids during handling, storage, transport or shipping;

1910.1030(d)(4)(iii)(B)(1)(iii)

Labeled or color-coded in accordance with paragraph (g)(1)(i) this standard; and

1910.1030(d)(4)(iii)(B)(1)(iv)

Closed prior to removal to prevent spillage or protrusion of contents during handling, storage, transport, or shipping.

1910.1030(d)(4)(iii)(B)(2)

If outside contamination of the regulated waste container occurs, it shall be placed in a second container. The second container shall be:

1910.1030(d)(4)(iii)(B)(2)(i)

Closable;

1910.1030(d)(4)(iii)(B)(2)(ii)

Constructed to contain all contents and prevent leakage of fluids during handling, storage, transport or shipping;

1910.1030(d)(4)(iii)(B)(2)(iii)

Labeled or color-coded in accordance with paragraph (g)(1)(i) of this standard; and

1910.1030(d)(4)(iii)(B)(2)(iv)

Closed prior to removal to prevent spillage or protrusion of contents during handling, storage, transport, or shipping.

1910.1030(d)(4)(iii)(C)

Disposal of all regulated waste shall be in accordance with applicable regulations of the United States, States and Territories, and political subdivisions of States and Territories.

1910.1030(d)(4)(iv)

Laundry.

1910.1030(d)(4)(iv)(A)

Contaminated laundry shall be handled as little as possible with a minimum of agitation.

1910.1030(d)(4)(iv)(A)(1)

Contaminated laundry shall be bagged or containerized at the location where it was used and shall not be sorted or rinsed in the location of use.

1910.1030(d)(4)(iv)(A)(2)

Contaminated laundry shall be placed and transported in bags or containers labeled or color-coded in accordance with paragraph (g)(1)(i) of this standard. When a facility utilizes Universal Precautions in the handling of all soiled laundry, alternative labeling or color-coding is sufficient if it permits all employees to recognize the containers as requiring compliance with Universal Precautions.

1910.1030(d)(4)(iv)(A)(3)

Whenever contaminated laundry is wet and presents a reasonable likelihood of soak-through or leakage from the bag or container, the laundry shall be placed and transported in bags or containers which prevent soak-through and/or leakage of fluids to the exterior.

1910.1030(d)(4)(iv)(B)

The employer shall ensure that employees who have contact with contaminated laundry wear protective gloves and other appropriate personal protective equipment.

1910.1030(d)(4)(iv)(C)

When a facility ships contaminated laundry off-site to a second facility which does not utilize Universal Precautions in the handling of all laundry, the facility generating the contaminated laundry must place such laundry in bags or containers which are labeled or color-coded in accordance with paragraph (g)(1)(i).

1910.1030(e)

HIV and HBV Research Laboratories and Production Facilities.

1910.1030(e)(1)

This paragraph applies to research laboratories and production facilities engaged in the culture, production, concentration, experimentation, and manipulation of HIV and HBV. It does not apply to clinical or diagnostic laboratories engaged solely in the analysis of blood, tissues, or organs. These requirements apply in addition to the other requirements of the standard.

1910.1030(e)(2)

Research laboratories and production facilities shall meet the following criteria:

1910.1030(e)(2)(i)

Standard Microbiological Practices. All regulated waste shall either be incinerated or decontaminated by a method such as autoclaving known to effectively destroy bloodborne pathogens.

1910.1030(e)(2)(ii)

Special Practices.

1910.1030(e)(2)(ii)(A)

Laboratory doors shall be kept closed when work involving HIV or HBV is in progress.

1910.1030(e)(2)(ii)(B)

Contaminated materials that are to be decontaminated at a site away from the work area shall be placed in a durable, leakproof, labeled or color-coded container that is closed before being removed from the work area.

1910.1030(e)(2)(ii)(C)

Access to the work area shall be limited to authorized persons. Written policies and procedures shall be established whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements, and who comply with all entry and exit procedures shall be allowed to enter the work areas and animal rooms.

1910.1030(e)(2)(ii)(D)

When other potentially infectious materials or infected animals are present in the work area or containment module, a hazard warning sign incorporating the universal biohazard symbol shall be posted on all access doors. The hazard warning sign shall comply with paragraph (g)(1)(ii) of this standard.

1910.1030(e)(2)(ii)(E)

All activities involving other potentially infectious materials shall be conducted in biological safety cabinets or other physical-containment devices within the containment module. No work with these other potentially infectious materials shall be conducted on the open bench.

1910.1030(e)(2)(ii)(F)

Laboratory coats, gowns, smocks, uniforms, or other appropriate protective clothing shall be used in the work area and animal rooms. Protective clothing shall not be worn outside of the work area and shall be decontaminated before being laundered.

1910.1030(e)(2)(ii)(G)

Special care shall be taken to avoid skin contact with other potentially infectious materials. Gloves shall be worn when handling infected animals and when making hand contact with other potentially infectious materials is unavoidable.

1910.1030(e)(2)(ii)(H)

Before disposal all waste from work areas and from animal rooms shall either be incinerated or decontaminated by a method such as autoclaving known to effectively destroy bloodborne pathogens.

1910.1030(e)(2)(ii)(I)

Vacuum lines shall be protected with liquid disinfectant traps and high-efficiency particulate air (HEPA) filters or filters of equivalent or superior efficiency and which are checked routinely and maintained or replaced as necessary.

1910.1030(e)(2)(ii)(J)

Hypodermic needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., the needle is integral to the syringe) shall be used for the injection or aspiration of other

potentially infectious materials. Extreme caution shall be used when handling needles and syringes. A needle shall not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The needle and syringe shall be promptly placed in a puncture-resistant container and autoclaved or decontaminated before reuse or disposal.

1910.1030(e)(2)(ii)(K)

All spills shall be immediately contained and cleaned up by appropriate professional staff or others properly trained and equipped to work with potentially concentrated infectious materials.

1910.1030(e)(2)(ii)(L)

A spill or accident that results in an exposure incident shall be immediately reported to the laboratory director or other responsible person.

1910.1030(e)(2)(ii)(M)

A biosafety manual shall be prepared or adopted and periodically reviewed and updated at least annually or more often if necessary. Personnel shall be advised of potential hazards, shall be required to read instructions on practices and procedures, and shall be required to follow them.

1910.1030(e)(2)(iii)

Containment Equipment.

1910.1030(e)(2)(iii)(A)

Certified biological safety cabinets (Class I, II, or III) or other appropriate combinations of personal protection or physical containment devices, such as special protective clothing, respirators, centrifuge safety cups, sealed centrifuge rotors, and containment caging for animals, shall be used for all activities with other potentially infectious materials that pose a threat of exposure to droplets, splashes, spills, or aerosols.

1910.1030(e)(2)(iii)(B)

Biological safety cabinets shall be certified when installed, whenever they are moved and at least annually.

1910.1030(e)(3)

HIV and HBV research laboratories shall meet the following criteria:

1910.1030(e)(3)(i)

Each laboratory shall contain a facility for hand washing and an eye wash facility which is readily available within the work area.

1910.1030(e)(3)(ii)

An autoclave for decontamination of regulated waste shall be available.

1910.1030(e)(4)

HIV and HBV production facilities shall meet the following criteria:

1910.1030(e)(4)(i)

The work areas shall be separated from areas that are open to unrestricted traffic flow within the building. Passage through two sets of doors shall be the basic requirement for entry into the work area from access corridors or other contiguous areas. Physical separation of the high-containment work area from access corridors or other areas or activities may also be provided by a double-doored clothes-change room (showers may be included), airlock, or other access facility that requires passing through two sets of doors before entering the work area.

1910.1030(e)(4)(ii)

The surfaces of doors, walls, floors and ceilings in the work area shall be water resistant so that they can be easily cleaned. Penetrations in these surfaces shall be sealed or capable of being sealed to facilitate decontamination.

1910.1030(e)(4)(iii)

Each work area shall contain a sink for washing hands and a readily available eye wash facility. The sink shall be foot, elbow, or automatically operated and shall be located near the exit door of the work area.

1910.1030(e)(4)(iv)

Access doors to the work area or containment module shall be self-closing.

1910.1030(e)(4)(v)

An autoclave for decontamination of regulated waste shall be available within or as near as possible to the work area.

1910.1030(e)(4)(vi)

A ducted exhaust-air ventilation system shall be provided. This system shall create directional airflow that draws air into the work area through the entry area. The exhaust air shall not be recirculated to any other area of the building, shall be discharged to the outside, and shall be dispersed away from occupied areas and air intakes. The proper direction of the airflow shall be verified (i.e., into the work area).

1910.1030(e)(5)

Training Requirements. Additional training requirements for employees in HIV and HBV research laboratories and HIV and HBV production facilities are specified in paragraph (g)(2)(ix).

1910.1030(f)

Hepatitis B Vaccination and Post-exposure Evaluation and Follow-up --

1910.1030(f)(1)

General.

1910.1030(f)(1)(i)

The employer shall make available the hepatitis B vaccine and vaccination series to all employees who have occupational exposure, and post-exposure evaluation and follow-up to all employees who have had an exposure incident.

1910.1030(f)(1)(ii)

The employer shall ensure that all medical evaluations and procedures including the hepatitis B vaccine and vaccination series and post-exposure evaluation and follow-up, including prophylaxis, are:

1910.1030(f)(1)(ii)(A)

Made available at no cost to the employee;

1910.1030(f)(1)(ii)(B)

Made available to the employee at a reasonable time and place;

1910.1030(f)(1)(ii)(C)

Performed by or under the supervision of a licensed physician or by or under the supervision of another licensed healthcare professional; and

1910.1030(f)(1)(ii)(D)

Provided according to recommendations of the U.S. Public Health Service current at the time these evaluations and procedures take place, except as specified by this paragraph (f).

1910.1030(f)(1)(iii)

The employer shall ensure that all laboratory tests are conducted by an accredited laboratory at no cost to the employee.

1910.1030(f)(2)

Hepatitis B Vaccination.

1910.1030(f)(2)(i)

Hepatitis B vaccination shall be made available after the employee has received the training required in paragraph (g)(2)(vii)(I) and within 10 working days of initial assignment to all employees who have occupational exposure unless the employee has previously received the complete hepatitis B vaccination series, antibody testing has revealed that the employee is immune, or the vaccine is contraindicated for medical reasons.

1910.1030(f)(2)(ii)

The employer shall not make participation in a prescreening program a prerequisite for receiving hepatitis B vaccination.

1910.1030(f)(2)(iii)

If the employee initially declines hepatitis B vaccination but at a later date while still covered under the standard decides to accept the vaccination, the employer shall make available hepatitis B vaccination at that time.

1910.1030(f)(2)(iv)

The employer shall assure that employees who decline to accept hepatitis B vaccination offered by the employer sign the statement in Appendix A.

1910.1030(f)(2)(v)

If a routine booster dose(s) of hepatitis B vaccine is recommended by the U.S. Public Health Service at a future date, such booster dose(s) shall be made available in accordance with section (f)(1)(ii).

1910.1030(f)(3)

Post-exposure Evaluation and Follow-up. Following a report of an exposure incident, the employer shall make immediately available to the exposed employee a confidential medical evaluation and follow-up, including at least the following elements:

1910.1030(f)(3)(i)

Documentation of the route(s) of exposure, and the circumstances under which the exposure incident occurred;

1910.1030(f)(3)(ii)

Identification and documentation of the source individual, unless the employer can establish that identification is infeasible or prohibited by state or local law;

1910.1030(f)(3)(ii)(A)

The source individual's blood shall be tested as soon as feasible and after consent is obtained in order to determine HBV and HIV infectivity. If consent is not obtained, the employer shall establish that legally required consent cannot be obtained. When the source individual's consent is not required by law, the source individual's blood, if available, shall be tested and the results documented.

1910.1030(f)(3)(ii)(B)

When the source individual is already known to be infected with HBV or HIV, testing for the source individual's known HBV or HIV status need not be repeated.

1910.1030(f)(3)(ii)(C)

Results of the source individual's testing shall be made available to the exposed employee, and the employee shall be informed of applicable laws and regulations concerning disclosure of the identity and infectious status of the source individual.

1910.1030(f)(3)(iii)

Collection and testing of blood for HBV and HIV serological status;

1910.1030(f)(3)(iii)(A)

The exposed employee's blood shall be collected as soon as feasible and tested after consent is obtained.

1910.1030(f)(3)(iii)(B)

If the employee consents to baseline blood collection, but does not give consent at that time for HIV serologic testing, the sample shall be preserved for at least 90 days. If, within 90 days of the exposure incident, the employee elects to have the baseline sample tested, such testing shall be done as soon as feasible.

1910.1030(f)(3)(iv)

Post-exposure prophylaxis, when medically indicated, as recommended by the U.S. Public Health Service;

1910.1030(f)(3)(v)

Counseling; and

1910.1030(f)(3)(vi)

Evaluation of reported illnesses.

1910.1030(f)(4)

Information Provided to the Healthcare Professional.

1910.1030(f)(4)(i)

The employer shall ensure that the healthcare professional responsible for the employee's Hepatitis B vaccination is provided a copy of this regulation.

1910.1030(f)(4)(ii)

The employer shall ensure that the healthcare professional evaluating an employee after an exposure incident is provided the following information:

1910.1030(f)(4)(ii)(A)

A copy of this regulation;

1910.1030(f)(4)(ii)(B)

A description of the exposed employee's duties as they relate to the exposure incident;

1910.1030(f)(4)(ii)(C)

Documentation of the route(s) of exposure and circumstances under which exposure occurred;

1910.1030(f)(4)(ii)(D)

Results of the source individual's blood testing, if available; and

1910.1030(f)(4)(ii)(E)

All medical records relevant to the appropriate treatment of the employee including vaccination status which are the employer's responsibility to maintain.

1910.1030(f)(5)

Healthcare Professional's Written Opinion. The employer shall obtain and provide the employee with a copy of the evaluating healthcare professional's written opinion within 15 days of the completion of the evaluation.

1910.1030(f)(5)(i)

The healthcare professional's written opinion for Hepatitis B vaccination shall be limited to whether Hepatitis B vaccination is indicated for an employee, and if the employee has received such vaccination.

1910.1030(f)(5)(ii)

The healthcare professional's written opinion for post-exposure evaluation and follow-up shall be limited to the following information:

1910.1030(f)(5)(ii)(A)

That the employee has been informed of the results of the evaluation; and

1910.1030(f)(5)(ii)(B)

That the employee has been told about any medical conditions resulting from exposure to blood or other potentially infectious materials which require further evaluation or treatment.

1910.1030(f)(5)(iii)

All other findings or diagnoses shall remain confidential and shall not be included in the written report.

1910.1030(f)(6)

Medical Recordkeeping. Medical records required by this standard shall be maintained in accordance with paragraph (h)(1) of this section.

1910.1030(g)

Communication of Hazards to Employees --

1910.1030(q)(1)

Labels and Signs --

1910.1030(q)(1)(i)

Labels.

1910.1030(g)(1)(i)(A)

Warning labels shall be affixed to containers of regulated waste, refrigerators and freezers containing blood or other potentially infectious material; and other containers used to store, transport or ship blood or other potentially infectious materials, except as provided in paragraph (g)(1)(i)(E), (F) and (G).

1910.1030(g)(1)(i)(B)

Labels required by this section shall include the following legend:



1910.1030(g)(1)(i)(C)

These labels shall be fluorescent orange or orange-red or predominantly so, with lettering and symbols in a contrasting color.

1910.1030(g)(1)(i)(D)

Labels shall be affixed as close as feasible to the container by string, wire, adhesive, or other method that prevents their loss or unintentional removal.

1910.1030(g)(1)(i)(E)

Red bags or red containers may be substituted for labels.

1910.1030(g)(1)(i)(F)

Containers of blood, blood components, or blood products that are labeled as to their contents and have been released for transfusion or other clinical use are exempted from the labeling requirements of paragraph (g).

1910.1030(g)(1)(i)(G)

Individual containers of blood or other potentially infectious materials that are placed in a labeled container during storage, transport, shipment or disposal are exempted from the labeling requirement.

1910.1030(g)(1)(i)(H)

Labels required for contaminated equipment shall be in accordance with this paragraph and shall also state which portions of the equipment remain contaminated.

1910.1030(g)(1)(i)(I)

Regulated waste that has been decontaminated need not be labeled or color-coded.

1910.1030(g)(1)(ii)

Signs.

1910.1030(g)(1)(ii)(A)

The employer shall post signs at the entrance to work areas specified in paragraph (e), HIV and HBV Research Laboratory and Production Facilities, which shall bear the following legend:



(Name of the Infectious Agent)

(Special requirements for entering the area)

(Name, telephone number of the laboratory director or other responsible person.)

1910.1030(g)(1)(ii)(B)

These signs shall be fluorescent orange-red or predominantly so, with lettering and symbols in a contrasting color.

[1910.1030\(g\)\(2\)](#)

Information and Training.

1910.1030(g)(2)(i)

The employer shall train each employee with occupational exposure in accordance with the requirements of this section. Such training must be provided at no cost to the employee and during working hours. The employer shall institute a training program and ensure employee participation in the program.

1910.1030(g)(2)(ii)

Training shall be provided as follows:

1910.1030(g)(2)(ii)(A)

At the time of initial assignment to tasks where occupational exposure may take place;

1910.1030(g)(2)(ii)(B)

At least annually thereafter.

1910.1030(g)(2)(iii)

[Reserved]

1910.1030(g)(2)(iv)

Annual training for all employees shall be provided within one year of their previous training.

1910.1030(g)(2)(v)

Employers shall provide additional training when changes such as modification of tasks or procedures or institution of new tasks or procedures affect the employee's occupational exposure. The additional training may be limited to addressing the new exposures created.

1910.1030(g)(2)(vi)

Material appropriate in content and vocabulary to educational level, literacy, and language of employees shall be used.

1910.1030(g)(2)(vii)

The training program shall contain at a minimum the following elements:

1910.1030(g)(2)(vii)(A)

An accessible copy of the regulatory text of this standard and an explanation of its contents;

1910.1030(g)(2)(vii)(B)

A general explanation of the epidemiology and symptoms of bloodborne diseases;

1910.1030(g)(2)(vii)(C)

An explanation of the modes of transmission of bloodborne pathogens;

1910.1030(g)(2)(vii)(D)

An explanation of the employer's exposure control plan and the means by which the employee can obtain a copy of the written plan;

1910.1030(g)(2)(vii)(E)

An explanation of the appropriate methods for recognizing tasks and other activities that may involve exposure to blood and other potentially infectious materials;

1910.1030(g)(2)(vii)(F)

An explanation of the use and limitations of methods that will prevent or reduce exposure including appropriate engineering controls, work practices, and personal protective equipment;

1910.1030(g)(2)(vii)(G)

Information on the types, proper use, location, removal, handling, decontamination and disposal of personal protective equipment;

1910.1030(g)(2)(vii)(H)

An explanation of the basis for selection of personal protective equipment;

1910.1030(g)(2)(vii)(I)

Information on the hepatitis B vaccine, including information on its efficacy, safety, method of administration, the benefits of being vaccinated, and that the vaccine and vaccination will be offered free of charge;

1910.1030(g)(2)(vii)(J)

Information on the appropriate actions to take and persons to contact in an emergency involving blood or other potentially infectious materials;

1910.1030(g)(2)(vii)(K)

An explanation of the procedure to follow if an exposure incident occurs, including the method of reporting the incident and the medical follow-up that will be made available;

1910.1030(g)(2)(vii)(L)

Information on the post-exposure evaluation and follow-up that the employer is required to provide for the employee following an exposure incident;

1910.1030(g)(2)(vii)(M)

An explanation of the signs and labels and/or color coding required by paragraph (g)(1); and

1910.1030(g)(2)(vii)(N)

An opportunity for interactive questions and answers with the person conducting the training session.

1910.1030(g)(2)(viii)

The person conducting the training shall be knowledgeable in the subject matter covered by the elements contained in the training program as it relates to the workplace that the training will address.

1910.1030(g)(2)(ix)

Additional Initial Training for Employees in HIV and HBV Laboratories and Production Facilities. Employees in HIV or HBV research laboratories and HIV or HBV production facilities shall receive the following initial training in addition to the above training requirements.

1910.1030(g)(2)(ix)(A)

The employer shall assure that employees demonstrate proficiency in standard microbiological practices and techniques and in the practices and operations specific to the facility before being allowed to work with HIV or HBV.

1910.1030(g)(2)(ix)(B)

The employer shall assure that employees have prior experience in the handling of human pathogens or tissue cultures before working with HIV or HBV.

1910.1030(g)(2)(ix)(C)

The employer shall provide a training program to employees who have no prior experience in handling human pathogens. Initial work activities shall not include the handling of infectious agents. A progression of work activities shall be assigned as techniques are learned and proficiency is developed. The employer shall assure that employees participate in work activities involving infectious agents only after proficiency has been demonstrated.

1910.1030(h)

Recordkeeping --

1910.1030(h)(1)

Medical Records.

1910.1030(h)(1)(i)

The employer shall establish and maintain an accurate record for each employee with occupational exposure, in accordance with 29 CFR 1910.1020.

1910.1030(h)(1)(ii)

This record shall include:

1910.1030(h)(1)(ii)(A)

The name and social security number of the employee;

1910.1030(h)(1)(ii)(B)

A copy of the employee's hepatitis B vaccination status including the dates of all the hepatitis B vaccinations and any medical records relative to the employee's ability to receive vaccination as required by paragraph (f)(2);

1910.1030(h)(1)(ii)(C)

A copy of all results of examinations, medical testing, and follow-up procedures as required by paragraph (f)(3);

1910.1030(h)(1)(ii)(D)

The employer's copy of the healthcare professional's written opinion as required by paragraph (f)(5); and

1910.1030(h)(1)(ii)(E)

A copy of the information provided to the healthcare professional as required by paragraphs (f)(4)(ii)(B)(C) and (D).

1910.1030(h)(1)(iii)

Confidentiality. The employer shall ensure that employee medical records required by paragraph (h)(1) are:

1910.1030(h)(1)(iii)(A)

Kept confidential; and

1910.1030(h)(1)(iii)(B)

Not disclosed or reported without the employee's express written consent to any person within or outside the workplace except as required by this section or as may be required by law.

1910.1030(h)(1)(iv)

The employer shall maintain the records required by paragraph (h) for at least the duration of employment plus 30 years in accordance with 29 CFR 1910.1020.

1910.1030(h)(2)

Training Records.

1910.1030(h)(2)(i)

Training records shall include the following information:

1910.1030(h)(2)(i)(A)

The dates of the training sessions;

1910.1030(h)(2)(i)(B)

The contents or a summary of the training sessions;

1910.1030(h)(2)(i)(C)

The names and qualifications of persons conducting the training; and

1910.1030(h)(2)(i)(D)

The names and job titles of all persons attending the training sessions.

1910.1030(h)(2)(ii)

Training records shall be maintained for 3 years from the date on which the training occurred.

1910.1030(h)(3)

Availability.

1910.1030(h)(3)(i)

The employer shall ensure that all records required to be maintained by this section shall be made available upon request to the Assistant Secretary and the Director for examination and copying.

[1910.1030\(h\)\(3\)\(ii\)](#)

Employee training records required by this paragraph shall be provided upon request for examination and copying to employees, to employee representatives, to the Director, and to the Assistant Secretary.

[1910.1030\(h\)\(3\)\(iii\)](#)

Employee medical records required by this paragraph shall be provided upon request for examination and copying to the subject employee, to anyone having written consent of the subject employee, to the Director, and to the Assistant Secretary in accordance with 29 CFR 1910.1020.

[1910.1030\(h\)\(4\)](#)

Transfer of Records. The employer shall comply with the requirements involving transfer of records set forth in 29 CFR 1910.1020(h).

[1910.1030\(h\)\(5\)](#)

Sharps injury log.

1910.1030(h)(5)(i)

The employer shall establish and maintain a sharps injury log for the recording of percutaneous injuries from contaminated sharps. The information in the sharps injury log shall be recorded and maintained in such manner as to protect the confidentiality of the injured employee. The sharps injury log shall contain, at a minimum:

[1910.1030\(h\)\(5\)\(i\)\(A\)](#)

The type and brand of device involved in the incident,

1910.1030(h)(5)(i)(B)

The department or work area where the exposure incident occurred, and

[1910.1030\(h\)\(5\)\(i\)\(C\)](#)

An explanation of how the incident occurred.

1910.1030(h)(5)(ii)

The requirement to establish and maintain a sharps injury log shall apply to any employer who is required to maintain a log of occupational injuries and illnesses under 29 CFR 1904.

1910.1030(h)(5)(iii)

The sharps injury log shall be maintained for the period required by 29 CFR 1904.6.

1910.1030(i)

Dates --

1910.1030(i)(1)

Effective Date. The standard shall become effective on March 6, 1992.

1910.1030(i)(2)

The Exposure Control Plan required by paragraph (c) of this section shall be completed on or before May 5, 1992.

1910.1030(i)(3)

Paragraph (g)(2) Information and Training and (h) Recordkeeping shall take effect on or before June 4, 1992.

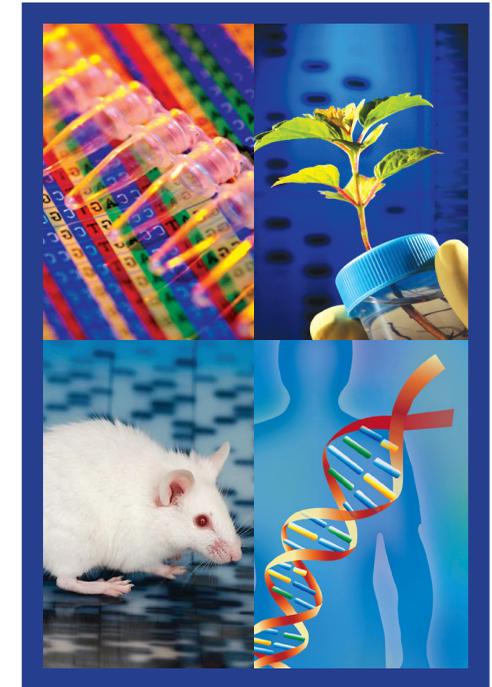
1910.1030(i)(4)

1910.1030(i)(4)

Paragraphs (d)(2) Engineering and Work Practice Controls, (d)(3) Personal Protective Equipment, (d)(4) Housekeeping, (e) HIV and HBV Research Laboratories and Production Facilities, (f) Hepatitis B Vaccination and Post-Exposure Evaluation and Follow-up, and (g)(1) Labels and Signs, shall take effect July 6, 1992.

[56 FR 64004, Dec. 06, 1991, as amended at 57 FR 12717, April 13, 1992; 57 FR 29206, July 1, 1992; 61 FR 5507, Feb. 13, 1996; 66 FR 5325 Jan., 18, 2001; 71 FR 16672 and 16673, April 3, 2006; 73 FR 75586, Dec. 12, 2008; 76 FR 33608, June 8, 2011]

Investigator Responsibilities



under the
**NIH Guidelines
for Research Involving
Recombinant DNA
Molecules**

PIs conducting human gene transfer research must:

- ◆ Ensure that all aspects of Appendix M have been appropriately addressed prior to submission of a human gene transfer experiment to NIH OBA for review by the NIH Recombinant DNA Advisory Committee (RAC).
- ◆ Provide a letter signed by the PI(s) on institutional letterhead acknowledging that the documentation being submitted to NIH OBA complies with the requirements set forth in Appendix M.
- ◆ Not enroll research participants in a human gene transfer experiment until the RAC review process has been completed; IBC approval (from the clinical trial site) has been obtained; Institutional Review Board approval has been obtained; and all applicable regulatory authorization(s) have been obtained.
- ◆ Comply with reporting requirements for human gene transfer experiments (see Appendix M-I-C of the *NIH Guidelines*).

For More Information

To receive updates on current initiatives, policies, and news from OBA, subscribe to our listserv, "OBA_NEWS," by sending a message to: listserv@list.nih.gov with the message: [subscribe OBA_NEWS](#)

Visit the following websites for additional information:

NIH Office of Biotechnology Activities
<http://oba.od.nih.gov>

**NIH Guidelines for Research Involving
Recombinant DNA Molecules**
[http://oba.od.nih.gov/rdna/
nih_guidelines_oba.html](http://oba.od.nih.gov/rdna/nih_guidelines_oba.html)

NIH Office of Biotechnology Activities
6705 Rockledge Drive, Suite 750
Bethesda, MD 20892-7985
Phone: 301-496-9838
Email: oba@od.nih.gov
Fax: 301-496-9839

What are the *NIH Guidelines for Research Involving Recombinant DNA Molecules*?

The *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* detail procedures and practices for the containment and safe conduct of various forms of recombinant DNA research, including research involving genetically modified plants and animals, and human gene transfer.

Who must comply with the *NIH Guidelines*?

All institutions that receive NIH funding for recombinant DNA research must comply with the *NIH Guidelines*. Researchers at institutions that are subject to the *NIH Guidelines* must comply with the requirements even if their individual projects are not funded by NIH.

What is an Institutional Biosafety Committee?

Institutional Biosafety Committees (IBCs) provide local review and oversight of nearly all forms of research utilizing recombinant DNA. They ensure that recombinant DNA research conducted at or sponsored by the institution is in compliance with the *NIH Guidelines*. A requirement of the *NIH Guidelines* is that an IBC must review and approve all research subject to the *NIH Guidelines*.

What is the NIH Office of Biotechnology Activities?

The NIH Office of Biotechnology Activities (OBA) promotes science, safety, and ethics in biotechnology through the advancement of knowledge, enhancement of public understanding, and development of sound public policies. A core responsibility of OBA is to foster awareness of, and adherence to, the standards and practices set forth in the *NIH Guidelines*.

***Safety and science
go hand in hand***

Principal Investigator Responsibilities

Principal Investigators (PIs) are responsible for full compliance with the *NIH Guidelines* during the conduct of recombinant DNA research. As part of this general responsibility, the PI should:

- ◆ Be adequately trained in good microbiological techniques.
- ◆ Provide laboratory research staff with protocols describing potential biohazards and necessary precautions.
- ◆ Instruct and train laboratory staff in: (i) the practices and techniques required to ensure safety, and (ii) the procedures for dealing with accidents.
- ◆ Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations or serum collection).
- ◆ Supervise laboratory staff to ensure that the required safety practices and techniques are employed.
- ◆ Correct work errors and conditions that may result in the release of recombinant DNA materials.
- ◆ Ensure the integrity of physical containment (e.g., biological safety cabinets) and biological containment (e.g., purity and genotypic and phenotypic characteristics).
- ◆ Comply with permit and shipping requirements for recombinant DNA molecules.
- ◆ Adhere to IBC-approved emergency plans for handling accidental spills and personnel contamination.

Before initiating research subject to the *NIH Guidelines*, the PI must:

- ◆ Determine whether the research is subject to Section III-A, III-B, III-C, III-D, or III-E of the *NIH Guidelines*.
- ◆ Propose physical and biological containment levels in accordance with the *NIH Guidelines* when registering research with the IBC.

- ◆ Propose appropriate microbiological practices and laboratory techniques to be used for the research.
- ◆ Submit a research protocol to the IBC for review and approval.
- ◆ Seek OBA's determination of containment for experiments that require case-by-case review.
- ◆ Petition OBA, with notice to the IBC, for proposed exemptions from the *NIH Guidelines*.
- ◆ Obtain IBC approval before initiating research subject to the *NIH Guidelines*.
- ◆ Seek NIH approval, in addition to IBC approval, to conduct experiments specified in Sections III-A and III-B of the *NIH Guidelines*.

While conducting research subject to the *NIH Guidelines*, the PI must:

- ◆ Determine the need for IBC review before modifying recombinant DNA research already approved by the IBC.
- ◆ Submit any subsequent changes (e.g., changes in the source of DNA or host-vector system) to the IBC for review and approval or disapproval.
- ◆ Remain in communication with the IBC throughout the duration of the project.
- ◆ Report any significant problems pertaining to the operation and implementation of containment practices and procedures, violations of the *NIH Guidelines*, or any significant research-related accidents and illnesses to the IBC, OBA, and, as applicable, the Biological Safety Officer, Greenhouse or Animal Facility Director, and other appropriate authorities.

Laboratories using biohazardous agents must have a biosafety manual available for immediate reference. Those labs using chemicals must also provide a chemical hygiene plan. The information in this template must be located in every lab and constitutes that lab's safety manual. Listed below are some suggestions for information that could be included in this manual. Regardless of format used, all information contained in this template must be included in the lab's manual.

Date _____

Principal Investigator _____

IBC Protocol No. _____

Containment Level: BSL1 ABSL2
 BSL2 ABSL3
 BSL3

Biohazardous Agent(s) Used:
(List all RG-2 and/or RG-3 agents)

Personal Protective Equipment Required:

Immunizations and/or Clinical Baseline Testing Required:

List of Trained Personnel:

Specific Procedure(s):

(Describe briefly any specialized procedures utilized in this laboratory that are not otherwise contained in the *NIH/CDC Biosafety Manual*. Additional sheets may be attached if necessary)

Emergency information:

(As applicable, provide information regarding emergency procedures and equipment specific to the lab(s) under your control. Include, evacuation procedures (e.g., close fire doors, secure certain equipment, etc.); first-aid kit (location contents, maintenance responsibility, etc.); spill cleanup materials (e.g., location, contents, maintenance, procedures, etc); (Lab monitors or alarms (e.g., operation, response, maintenance, etc).

Material Safety Data Sheets (MSDS):

Per OSHA, all lab chemical users must know: a) what and MSDS is, B) MSDS relevance to their health and safety, (c) where they are located and how to readily access them. Labs are encouraged to maintain their own MSDS for the hazardous chemicals they routinely use.

SELECT AGENTS AND TOXINS

The following biological agents and toxins have been determined to have the potential to pose a severe threat to both human and animal health, to plant health, or to animal and plant products. An attenuated strain of a select agent or an inactive form of a select toxin may be excluded from the requirements of the Select Agent Regulations. The list of excluded agents and toxins can be found at: <http://www.selectagents.gov/Select%20Agents%20and%20Toxins%20Exclusions.html>

HHS SELECT AGENTS AND TOXINS

Abrin
Botulinum neurotoxins
Botulinum neurotoxin producing species of *Clostridium*
Cercopithecine herpesvirus 1 (Herpes B virus)
Clostridium perfringens epsilon toxin
Coccidioides posadasii/Coccidioides immitis
Conotoxins
Coxiella burnetii
Crimean-Congo haemorrhagic fever virus
Diacetoxyscirpenol
Eastern Equine Encephalitis virus
Ebola virus
Francisella tularensis
Lassa fever virus
Marburg virus
Monkeypox virus
Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments (Reconstructed 1918 Influenza virus)
Ricin
Rickettsia prowazekii
Rickettsia rickettsii
Saxitoxin
Shiga-like ribosome inactivating proteins
Shigatoxin
South American Haemorrhagic Fever viruses
Flexal
Guanarito
Junin
Machupo
Sabia
Staphylococcal enterotoxins
T-2 toxin
Tetrodotoxin
Tick-borne encephalitis complex (flavi) viruses
Central European Tick-borne encephalitis
Far Eastern Tick-borne encephalitis
Kyasanur Forest disease
Omsk Hemorrhagic Fever
Russian Spring and Summer encephalitis
Variola major virus (Smallpox virus)
Variola minor virus (Alastrim)
Yersinia pestis

OVERLAP SELECT AGENTS AND TOXINS

Bacillus anthracis
Brucella abortus
Brucella melitensis
Brucella suis
Burkholderia mallei (formerly *Pseudomonas mallei*)
Burkholderia pseudomallei (formerly *Pseudomonas pseudomallei*)
Hendra virus
Nipah virus
Rift Valley fever virus
Venezuelan Equine Encephalitis virus

USDA VETERINARY SERVICES (VS) SELECT AGENTS

African horse sickness virus
African swine fever virus
Akabane virus
Avian influenza virus (highly pathogenic)
Bluetongue virus (exotic)
Bovine spongiform encephalopathy agent
Camel pox virus
Classical swine fever virus
Ehrlichia ruminantium (Heartwater)
Foot-and-mouth disease virus
Goat pox virus
Japanese encephalitis virus
Lumpy skin disease virus
Malignant catarrhal fever virus
(Alcelaphine herpesvirus type 1)
Menangle virus
Mycoplasma capricolum subspecies *capripneumoniae* (contagious caprine pleuropneumonia)
Mycoplasma mycoides subspecies *mycoides* small colony (*MmmSC*) (contagious bovine pleuropneumonia)
Peste des petits ruminants virus
Rinderpest virus
Sheep pox virus
Swine vesicular disease virus
Vesicular stomatitis virus (exotic): Indiana subtypes
VSV-IN2, VSV-IN3
Virulent Newcastle disease virus¹

USDA PLANT PROTECTION AND QUARANTINE (PPQ) SELECT AGENTS

Peronosclerospora philippinensis (*Peronosclerospora sacchari*)
Phoma glycinicola (formerly *Pyrenochaeta glycines*)
Ralstonia solanacearum race 3, biovar 2
Rathayibacter toxicus
Sclerophthora rayssiae var. *zeae*
Synchytrium endobioticum
Xanthomonas oryzae
Xylella fastidiosa (citrus variegated chlorosis strain)

9/19/11

¹ A virulent Newcastle disease virus (avian paramyxovirus serotype 1) has an intracerebral pathogenicity index in day-old chicks (*Gallus gallus*) of 0.7 or greater or has an amino acid sequence at the fusion (F) protein cleavage site that is consistent with virulent strains of Newcastle disease virus. A failure to detect a cleavage site that is consistent with virulent strains does not confirm the absence of a virulent virus.

<input type="checkbox"/> New Submission	<input type="checkbox"/> Resubmission	IBC Protocol #	
<input type="checkbox"/> Amendment	<input type="checkbox"/> 1-Year Renewal		

General Information

(To be completed for all recombinant and biohazardous materials use and experimentation)

Important note: All investigators and participating personnel must have documentation showing participation in the approved Biosafety Training provided by Biosafety Officers of KCUMB. The investigators should provide up to three (3) names and contact information of the potential experts who provide expert opinion/critiques about the application.

General Biosafety as well as lab-specific training is required for all participants in accordance with KCUMB policy.

Project Title		
Principal Investigator: Name	Degree	Position/Title
Address	Phone	Email
Location(s) of Research - Bldg/Rm(s)	IRB, IACUC, or Other Protocol #s (for reference)	

Secondary or Co-Investigators

Name	Degree	Position/Title	Address, Phone, Email

Research Personnel

Name	Position/Title	Role in Research Protocol	Address, Phone, Email

Is this Project Funded? Yes No

Name of Funding Agency _____

The title of the Funding Project and project number (if available) _____

The signature certifies that the PI understands and accepts the following obligations in this study:

- I recognize that as the PI it is my responsibility to ensure that this research and the actions of all project personnel involved in conducting the study will conform with the IBC approved protocol and the provisions of the *NIH Guidelines for Research Involving Recombinant DNA*, the *CDC/NIH Biosafety in Microbiological and Biomedical Laboratories* manual, and the *Select Agent Rule* where appropriate. I will inform the IBC of any unanticipated biosafety related problems encountered while doing the research.
- I will notify the IBC of any change in a BSL-1 protocol.
- I will not initiate any change in a BSL-2 or BSL-3 protocol *without prior IBC approval*.
- *I will maintain all required research records on file and I recognize that representatives of the IBC are authorized to inspect these records periodically.*
- I accept responsibility for the safe conduct of the experiments to be conducted and will see that all associated personnel are trained in the safe laboratory practices required for this work.
- I will oversee the development and implementation of standard biosafety operating procedures for the laboratory.
- I understand that IBC approval is valid for **1 year** and an annual IBC update is required in order to maintain approved status.
- I accept responsibility that all personnel working in my laboratory will be trained to report any biological spill to me and that any spills involving the contamination of personnel and/or the environment that has the potential to cause illness or may cause sufficient concern to the public will be reported to the IBC within the regulatory deadlines specified.
- I will instruct employees to report to me, or in my absence to Biological Safety, Occupational health and Infectious Disease, any infection where a potential exists that the infection may have been occupationally acquired.
- I understand that failure to comply with all NIH regulations, IBC requirements/policies, and the provisions of the protocol as approved by the IBC may result in suspension or termination of my research project.

For protocols involving the use of Select Agents (as defined in 42 CFR Part 73):

- I will comply with the requirements for the reporting and securing of select agents that fall within the bounds of 42 CFR Part 73.

For protocols using animals in research:

- I will contact the IACUC staff and develop standard operating procedures (SOPs) and an IACUC protocol to address relevant operational biocontainment and safety issues for the use of these agents in animals, prior to their introduction into animals. IBC protocol approval is required prior to IACUC protocol approval.

Signature of PI

Date

Signature of Department Head

Date

For Biological Safety Committee Office Use Only

The Biological Safety Committee has reviewed the proposed project and has found it to be in compliance with the *NIH Guidelines*, the *CDC/NIH Biosafety in Microbiological and Biomedical Laboratories* manual, and the *Select Agent Rule* requirement for research involving biohazardous materials.

Biosafety Officer Signature

Approved Biosafety Level
Date
Protocol Approval Date
1 Year Expiration Date
Chair Signature

Yes	No	Type of Biohazardous Material (Check all that apply).
		Recombinant DNA (Exempt and Non-Exempt)
		Biosafety Level 2 Infectious Agents, Select Agents, and Biological Toxins
		Biosafety Level 3 Infectious Agents, Select Agents, and Biological Toxins
		Blood Borne Pathogens, Human Blood, Fluids, Tissues, Human Origin Cell Lines, and other Potentially Infectious Material (OPIM)
		Human Gene Transfer
		USDA and CDC Regulated Agents
		Dual Use Research concerns

Scientific Summary and Rationale

Provide a concise description of the work proposed with enough detail for the IBC to evaluate the safety protocols.

Do not copy grant application sections. Explain only experiments that will be performed under *this* protocol. It is not necessary to supply kit manuals; if they are useful for the committee a link to the on-line version is acceptable. If more space is needed the text box will expand indefinitely or please attach the information.

Risk Assessment

1. Indicate the risk group (or class) of all material(s) used in the experiments performed under this protocol. For a searchable database of risk groups go to <http://www.absa.org/riskgroups/index.html>.

	Risk Group 1	Agents are <i>NOT</i> associated with disease in healthy adult humans.
	Risk Group 2	Agents are associated with human disease that is rarely serious. There are often preventive or therapeutic interventions available.
	Risk Group 3	Agents are associated with serious or lethal human disease for which preventive or therapeutic interventions <i>MAY</i> be available.
	Risk Group 4	Agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are <i>NOT USUALLY</i> available.

2. Indicate the biosafety level(s) at which recombinant DNA and/or biohazardous experiments will be performed.

	BSL-1	Low risk agents (generally risk group 1), special containment equipment not required <ul style="list-style-type: none"> • Work is done on open bench tops. • Standard microbiological practices are observed Biohazard signs should be posted.
	BSL-2	Moderate risk agents (generally risk group 2), biosafety cabinets, restrictions to research areas. All BSL-1 containment and practices plus the following: <ul style="list-style-type: none"> • Laboratory access is restricted when experimental work is in progress. • Personnel have specific training in handling of agents. • Biological safety cabinets (BSC) or other physical containment devices are used for potential aerosol generation procedures. • Biohazard signs must be posted. • Specific PPE (personnel protective equipment) and entrance requirements.
	BSL-3	High risk agents (generally risk group 3), BSL-3 containment facilities, and practices. All BSL-2 containment and practices plus the following: <ul style="list-style-type: none"> • Laboratory access is restricted to unrelated/untrained investigators and associates • Personnel have specific training in handling of agents. • All procedures are performed in biological safety cabinets (BSC). • Biohazard signs must be posted. • Written safety policies provided by the investigator defining laboratory procedures, waste disposal, disinfection and medical surveillance. • Centrifuge safety cups must be used. • Specific facility design parameters must be followed, including requirements for location, ventilation, room integrity and security.

3. Is the highest proposed Biosafety Level lower or higher than the risk group classification for any of the described agents? (e.g. use of a single gene from a risk group 3 organism may qualify for recombinant work at BSL-1 or BSL-2).

	Yes
	No

If "Yes", explain the rationale for the use of the lower/higher Biosafety Level.

4. Describe the potential biosafety risks of this research proposal. Address the following issues:
- Are agent(s) used that may be infectious to humans?
 - Describe the risks of accidental exposure to personnel.
 - Does the potential exist for airborne transmission of agent(s)?
 - Describe precautions to be taken by personnel including any personal protective equipment and/or routine monitoring.
 - Describe disposal of agent(s) including methods for disposal or inactivation of agent(s) and of contaminated or infectious material(s).

Note that specific *detailed answers* are requested in Appendix A-1 (SECTIONS B, C, D and E), A-2 and/or A-3, depending on the experiments proposed and the biohazardous materials and agents involved.

--

5. Describe the Principal Investigator's experience with procedures and experiments described for this application including the use of agents, organisms, viruses, vectors and/or recombinant DNA materials and procedures. Include the relevant publications and citations, if any

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Dual Use Research Concerns

6. The potential of Dual Use Research, research with a identifiable benefit but with the potential for misuse, has become a public safety concern that is being regulated at the federal level based on NSABB considerations and local oversight responsibility resides with the IBC.

If any of the questions posed below for research covered by this IBC application are answered by 'Yes', please attach as a separate page your description of the Dual Use aspects of the project

The IBC website at www.kcumb.edu/ibc Biosafety Training - Presentations provides background for these questions.

- Will this work create resistance to therapeutically useful antimicrobials or antivirals? (This applies to therapeutic agents used to control disease agents in humans, animals or plants)
- Will this work enhance the virulence of a pathogen or render a non-pathogen virulent? (This applies to human, animal or plant pathogens)
- Will this work increase the transmissibility of a pathogen? (This would include enhancing transmission within or between species, and altering vector competence to enhance disease transmission)
- Will this work alter the host range of a pathogen?
- Could the results of the project potentially demonstrate how to render a vaccine ineffective? (This applies to both human and animal vaccines)
- Could this work enable the evasion of diagnostic/detection modalities? (e.g., microencapsulation to avoid antibody-based detection and/or the alteration of gene sequences to avoid detection by established molecular methods)
- Could this work enable the weaponization of a pathogen or toxin (e.g., stabilization or synthesis of a "high-risk" agent)

	Yes
	No

In responding in a free-form attachment to a 'Yes' answer, please consider not only the Project Design issues but also Research Progress and Completion issues ([www.kcumb.edu /ibc/docs/Dual-Use Considerations.pdf](http://www.kcumb.edu/ibc/docs/Dual-Use%20Considerations.pdf))

OUTCOME OF REVIEW (IBC USE ONLY)		
<input type="checkbox"/> Approval	<input type="checkbox"/> Modifications Required to Secure Approval (Explain in comments).	<input type="checkbox"/> Approval Withheld
IBC Comments:		
IBC Chair (print)		Date
Signature		

* IRBNet electronic signatures may be used in lieu of signing a hard copy of this document.

SECTION A

a1. The proposed experiments with recombinant DNA molecules are (check one):

<input type="checkbox"/> EXEMPT under the NIH Guidelines (go to question a2)	<input type="checkbox"/> Non-Exempt according to the NIH Guidelines (go to question a3)
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Criteria why your experiments may be considered **EXEMPT** recombinant research.

- If any experiments involving rDNA molecules are not fully within the categories described below at a2, they are by definition “Non-Exempt”. If this applies, continue with question a3.

a2. If you checked “Exempt” in question 1, indicate under which criteria the experiments are exempt by marking the unshaded boxes.

A.		The experiments involve rDNA molecules that are not in organisms or viruses (Section III-F-1 of the NIH Guidelines).
B.		The experiments involve rDNA molecules that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent (Section III-F-2 of the NIH Guidelines).
C.		The experiments involve rDNA molecules that consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well established physiological means (Section III-F-3 of the NIH Guidelines).
D.		The experiments involve rDNA molecules that consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species) (Section III-F-4 of the NIH Guidelines).
E.		The experiments involve rDNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent (Section III-F-5 of the NIH Guidelines).
F.		The experiments involve rDNA molecules that do not present a significant risk to health or the environment as determined by the NIH Director (Section III-F-6 of the NIH Guidelines).
F-1		<u>Recombinant DNA in Tissue Culture</u> . Experiments involve rDNA molecules containing less than one-half of any eukaryotic viral genome (all viruses from a single family being considered identical), that are propagated and maintained in cells in tissue culture (Appendix C-I-A of the NIH Guidelines).
F-2		<u>Escherichia coli K-12 Host-Vector Systems</u> . Experiments which use <i>E. coli</i> K-12 host-vector systems, with the exception of those experiments listed in Appendix C-II-A of the NIH Guidelines, are exempt provided that: (i) the <i>E. coli</i> host does not contain conjugation proficient plasmids or generalized transducing phages; or (ii) lambda or lambdoid or Ff bacteriophages or non-conjugative plasmids shall be used as vectors. Experiments involving the insertion into <i>E. coli</i> K-12 of DNA from prokaryotes that exchange genetic information with <i>E. coli</i> may be performed with any <i>E. coli</i> K-12 vector (e.g., conjugative plasmid).
F-3		<u>Saccharomyces Host-Vector Systems</u> . Experiments involving <i>Saccharomyces cerevisiae</i> and <i>Saccharomyces uvarum</i> host-vector systems are exempt from the <i>NIH Guidelines</i> , with the exception of experiments listed in Appendix C-III-A,

NOTE that this table continues on the next page

F-4	<u>Bacillus subtilis</u> or <u>Bacillus licheniformis</u> Host-Vector Systems. Any asporogenic <i>Bacillus subtilis</i> or asporogenic <i>Bacillus licheniformis</i> strain which does not revert to a sporeformer with a frequency greater than 10^{-7} may be used for cloning DNA with the exception of those experiments listed in Appendix C-IV-A of the NIH Guidelines.
F-5	<u>Extrachromosomal Elements of Gram Positive Organisms</u> Recombinant DNA molecules derived entirely from extrachromosomal elements of the organisms listed in Appendix C-V of the NIH Guidelines (including shuttle vectors constructed from vectors described in Appendix C), propagated and maintained in organisms listed in those Guidelines are exempt.
F-6	<u>Purchase or Transfer of Transgenic Rodents.</u> The purchase or transfer of transgenic rodents for experiments requiring Biosafety Level 1 (BSL1) containment are exempt. (Appendix C-VI as described in Appendix GIII-M of the NIH Guidelines).
F-7	<u>Generation of BSL-1 Transgenic Rodents via Breeding.</u> The breeding of two different transgenic rodents or the breeding of a transgenic rodent and a non-transgenic rodent with the intent of creating a new strain of transgenic rodent that can be housed at BSL-1 containment will be exempt from the NIH Guidelines if : (1) Both parental rodents can be housed under BSL-1 containment; and (2) neither parental transgenic rodent contains the following genetic modifications: i. incorporation of more than one-half of the genome of an exogenous eukaryotic virus from a single family of viruses; or ii. incorporation of a transgene that is under the control of a gammaretroviral long terminal repeat (LTR); and (3) the transgenic rodent that results from this breeding is not expected to contain more than one-half of an exogenous viral genome from a single family of viruses.

- If any experiments involving rDNA molecules are not fully within the categories described above at a2, they are by definition "Non-Exempt". If this applies, continue with question a3.

Criteria that define the classification of **NON-EXEMPT** recombinant research.

❖ *Inclusion of Appendix A-1 for 'Non-Exempt' recombinant research requires completion by all listed researchers of the CITI IBC courses "Training for Investigators, Staff and Students Handling Biohazards" and "NIH Recombinant DNA (rDNA) Guidelines".*

a3.	If you checked "Non-Exempt" in question a1, indicate under which criteria the experiments are non-exempt by marking the unshaded boxes for the appropriate registration category (or categories) for experiments covered by <i>NIH Guidelines</i> . For a searchable database of risk groups go to http://www.absa.org/riskgroups/index.html .
A	<i>Experiments that require NIH Director approval, Recombinant DNA Advisory Committee (RAC) review, and IBC approval (NIH Guidelines, Section III-A)</i>
<input type="checkbox"/>	Major Actions under the <i>NIH Guidelines</i>
B	<i>Experiments that require NIH/OBA and IBC approval (NIH Guidelines, Section III-B)</i>
<input type="checkbox"/>	Experiments involving the cloning of toxin molecules with LD50 of less than 100 nanograms per kilogram body weight

NOTE that this table continues on the next page

C	<i>Experiments that require IBC approval and Institutional Review Board (IRB) approval (NIH Guidelines, Section III-C)</i>
	<p>Experiments involving the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into one or more human research participants (human gene transfer) (<i>NIH Guidelines, Section III-C-1</i>)</p> <p>❖ <i>Human gene transfer research requires completion by all listed researchers of the CITI IBC course "Human Gene Transfer Trials".</i></p>
D	<i>Experiments that require IBC approval before initiation (NIH Guidelines, Section III-D)</i>
	Experiments using Risk Group 2, 3, 4 or restricted agents as host-vector systems (<i>NIH Guidelines, Section III-D-1</i>).
	Experiments in which DNA from Risk Group 2, 3, 4 or restricted agents is cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector systems (<i>NIH Guidelines, Section III-D-2</i>)
	Experiments involving the use of infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of helper virus in tissue culture systems (<i>NIH Guidelines, Section III-D-3</i>)
	Experiments involving whole animals requiring BSL-2, 3 or 4 containment (experiments with and breeding transgenic animals, and experiments involving viable recombinant DNA-modified microorganisms tested on whole animals) (<i>NIH Guidelines, Section III-D-4</i>).
	Experiments involving whole plants (to genetically engineer plants by recombinant DNA methods) (<i>NIH Guidelines, Section III-D-5</i>)
	Experiments using more than 10 liters of culture (<i>NIH Guidelines, Section III-D-6</i>)
	Experiments involving Influenza viruses (<i>NIH Guidelines, Section III-D-7</i>)
E	<i>Experiments that require IBC notice simultaneous with initiation. (NIH Guidelines, Section III-E)</i>
	Experiments involving the formation of recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (<i>NIH Guidelines, Section III-E-1</i>)
	Experiments involving whole plants (using DNA-modified whole plants and/or DNA-modified organisms associated with whole plants) (<i>NIH Guidelines, Section III-E-2</i>)
	Experiments involving transgenic rodents at BSL-1 containment – cross breeding different strains of transgenic or knockout rodents. (<i>NIH Guidelines, Section III-E-3</i>)
<i>Experiments not included in NIH Guidelines Sections III-A, III-B, III-C, III-D and III-F, and their subsections are considered in Section III-E.</i>	
	<p>IF your experiments fit neither one-or-more of the specific 'Exempt' or 'Non-Exempt' categories, the NIH Guidelines define any such research as subject to Section III-E.</p> <p>Explain below which experimental protocols are, for this reason, identified as III-E "Non-Exempt":</p>
<div style="border: 1px solid black; height: 28px;"></div>	

SECTION B – Vectors, Hosts, and rDNA Agents

b1. List all plasmid, phage and other bacterial and eukaryotic cell vectors used.
Enter "NA" if none.
(Note: viruses and viral vectors are listed in SECTION C.)

b2. Identify DNA sequences that will be inserted into vectors listed in b1. Include:

- the **species** from which the insert is derived;
- the gene product that is expressed, if any.

Enter "No insert" if no inserts are used.

b3. List known oncogenes, or inserts listed in b2 that have oncogenic properties.
Enter "NA" if no oncogene sequences are used.

b4. List host organisms for recombinant DNA transformations (e.g. *E.coli*, *S.cerevisiae*, other fungi, any plants, mammalian cells or human cell lines). List all metazoan vertebrate *and* invertebrate host species, including insects and worms.
(More information related to use of animals as rDNA hosts is requested in SECTION D.)
(Note: Recipient hosts for transient transfections need not to be listed. This type of experiment is currently not subject to NIH Guidelines for Research Involving Recombinant DNA Molecules.)

b5. List any sequences used to manipulate gene function (e.g. siRNA, microRNA) or as adjuvants (e.g. CpG-containing DNA) either in cell culture or *in vivo*. Enter "NA" if none are used.
(Note: *In vitro* creation and manipulation of nucleotide sequences, e.g. by PCR, if not propagated in cells, is currently not subject to NIH Guidelines for Research Involving Recombinant DNA Molecules.)

b6. Are human toxins to be expressed and released as part of this research?
Note: In case of significant human toxicity, BSL-2- and/or BSL-3-related Appendix A-2 and/or Appendix A-3 must be completed.

	Yes
	No

If "Yes", describe the toxic product(s) (including the LD₅₀) that could be produced or released and the containment precautions that will be used.

SECTION C – Viruses and virus vectors

- c1.** • Does this project involve the use of viruses or viral vectors?
(For bacterial and eukaryotic plasmid-like vectors, see SECTION B.)

<input type="checkbox"/>	Yes
<input type="checkbox"/>	No

If “No”, skip to SECTION D.

- c2.** List all viruses and/or viral vectors used:
- Specify the virus family and/or subfamily (e.g. herpesvirus, oncogenic retrovirus, adenovirus, adeno-associated virus, etc).
 - State the species of origin for each virus or vector used.

--

- For lentiviruses (e.g. FIV, HIV, SIV, etc) and lentiviral vectors, complete ONLY questions c7 – c12.
- For all other viruses, complete ONLY questions c3 – c6.

- c3.** Is the virus/viral vector able to enter or infect human cells?

<input type="checkbox"/>	Yes
<input type="checkbox"/>	No

If “Yes”, indicate whether it is a productive or limited infection, and state whether infection can cause disease.

--

- c4.** Is a helper virus used in this project?

<input type="checkbox"/>	Yes
<input type="checkbox"/>	No

If “Yes”, describe the helper virus used.

--

- c5.** Is the virus/viral vector replication-defective?

<input type="checkbox"/>	Yes
<input type="checkbox"/>	No

If “No”, skip question c6.

If “Yes”, describe the deletions rendering it defective, and complete question c6.

--

- c6.** Has the preparation of replication-defective vectors been tested for the presence of replication competent virus?

<input type="checkbox"/>	Yes
<input type="checkbox"/>	No

If "Yes", provide details of the assay used.
If "No", what is the likelihood of conversion to replication-competent virus?

--

For viruses other than lentiviruses, skip to SECTION D.

c7. List the specific lentivirus or strain and species of origin (e.g. HIV, human; FIV, feline).

--

c8. Is the lentivirus/lentiviral vector obtained from a commercial source?

Yes
No

If "Yes", provide the name of the commercial source.
If "No", provide the source of the lentivirus/lentiviral vector (e.g. the name of the institution or individual supplying the material).

--

c9. Is the lentivirus/lentiviral vector generated from a multi-component system (e.g. separate plasmids for packaging, envelope and gene transfer)?

Yes
No

If "Yes", describe the system used.

--

c10. Is the lentivirus/lentiviral vector pseudotyped (e.g. expressing a different envelope gene)?

Yes
No

If "Yes", provide whether the pseudotyping alters the host and cell tropism.

--

c11. Is the lentivirus/lentiviral vector replication-defective?

Yes
No

If "No", skip to SECTION D.
If "Yes", describe the deletions rendering it defective and complete question c12.

--

SECTION D – Animal Use Information

**Note that under NIH Recombinant Guidelines all metazoan species are included in this section.
This includes the use of insects, worms, molluscs and any vertebrate species.**

- ❖ *Research using small mammals requires completion by all listed researchers of the CITI IBC course "Animal Biosafety".*

d1. Does the work involve animal use?

Yes

No

If "Yes", list all animal species and strains

If "No", skip to SECTION E

d2. Identify all recombinant sequences and their associated biological risk.

d3. Do the experiments involve breeding of any transgenic animals, other than BSL-1 rodents?
Note, this includes the use of breeding for maintenance of strains. (Only strain maintenance and cross-breeding of BSL-1 transgenic rodents is generally Exempt (see section A, a2, F-7 above).)
Breeding of any other transgenic animals or of transgenic rodents requiring BSL-2+ containment is never Exempt.

Yes

No

If "Yes", please provide detailed information about breeding strategies and associated animal Biosafety concerns.

If "No", skip to SECTION E

d4. Is rodent breeding for transgenic strain maintenance performed, including maintaining Knock-Out strains?

Yes

No

If "Yes", describe associated animal BSL containment (ABSL1, ABSL2 or ABSL3) and the basis for this containment.

d5. Is rodent breeding performed to create new combinations of transgenic strains (e.g. congenics), including breeding of transgenic to non-transgenic/non recombinant lines, that will result in the creation or maintenance of strains which are NOT Exempt as specified in Section A, a2, F-7 ?

<input type="checkbox"/>
<input type="checkbox"/>

Yes
No

If "Yes", list the strains being bred and/or cross-bred:

	X	
	X	
	X	
	X	
	X	
	X	
	X	
	X	
	X	

Explain the rationale for this breeding program, and the intended use of the offspring. Describe the pertinent details of the experiment, including BSL containment requirements, the presence of exogenous eukaryotic viruses and/or the use of gammaretroviral LTRs.

--

d6. Has an Institutional Animal Care and Use Committee (IACUC) application been submitted?

<input type="checkbox"/>
<input type="checkbox"/>

Yes
No

If "Yes", provide the IACUC protocol number to be linked to this rDNA project.

NOTE: REGISTRATION OF THE PROTOCOL WITH AND APPROVAL BY THE IACUC IS REQUIRED BEFORE THE RESEARCH CAN BE INITIATED.

--

d7. Will recombinant agents be administered to live or intact animals? (e.g. application of plasmids, viral vectors, transfected cells, recombinant stem cells, etc.)

<input type="checkbox"/>
<input type="checkbox"/>

Yes
No

If "Yes", list agents.

If "No", skip to SECTION E.

--

d8. Do you anticipate that work with animal subjects will be conducted at a different BioSafety Level than any *in vitro* portions of the study?

<input type="checkbox"/>
<input type="checkbox"/>

Yes
No

If "Yes", provide the BSL for work with or housing of animal subjects, and explain the rationale or justification for the proposed BSL.

d9. Describe the route of administration for each recombinant agent used.

d10. Provide the concentration and volume for each recombinant agent to be administered

SECTION E – Human Use Information

(Note: use of human cells, cell lines and tissues requires at least BSL-2 containment: see Appendix A-4.)
ALL questions in Section E must be answered; none can be left blank.

e1.	Does work involve human cell lines (including cell lines such as 293T, HeLa)?
<input type="checkbox"/>	Yes
<input type="checkbox"/>	No
If "Yes", list below.	

e2.	Will primary human tissues or cells be used?
<input type="checkbox"/>	Yes
<input type="checkbox"/>	No
If "Yes", describe the use of the tissues or cells	

e3.	Has an Institutional Review Board (IRB) application been submitted?
<input type="checkbox"/>	Yes
<input type="checkbox"/>	No
If "Yes", provide the IRB protocol number to be linked to this rDNA project. NOTE: REGISTRATION OF THE PROTOCOL WITH AND APPROVAL BY THE IRB IS REQUIRED BEFORE THE RESEARCH CAN BE INITIATED.	

e4.	Is this a human DNA transfer proposal, i.e. is DNA and/or RNA derived from recombinant DNA administered to human subjects?
<input type="checkbox"/>	Yes
<input type="checkbox"/>	No
If "Yes", you must submit a detailed addendum in which each topic of Appendix M in the NIH Guidelines for Research Involving Recombinant DNA Molecules is addressed. PATIENT CONSENT FORMS, PROOF OF SUBMISSION OF PROPOSAL TO NIH, AND RAC APPROVAL MUST ALSO BE SUBMITTED.	

*This form is to be submitted on IRBNet when applicable by the Principal Investigator. Submission of this form on IRNet constitutes the signature of the Principal Investigator and his or her agreement that the information provided is accurate.

**NOTICE PERTINENT TO THE OCTOBER 2011 REVISIONS
OF THE
NIH GUIDELINES FOR RESEARCH INVOLVING
RECOMBINANT DNA MOLECULES
(NIH GUIDELINES)**

Summary of Amendments:

Minor Actions under [Section IV-C-1-b-\(2\)-\(c\)](#): Revising the *Classification of Etiologic Agents* for the purpose of these *NIH Guidelines* (see [Section V-A](#), *Footnotes and References of Sections I-IV*).

- Page 13: [Section II-A-3](#) – First paragraph, last sentence: Added a reference to [Appendix B: Classification of Human Etiologic Agents on The Basis of Hazard](#).
- Page 39: [Appendix B-II-A](#):
Added: “--*Coxiella burnetii* – specifically the Phase II, Nine Mile strain, plaque purified, clone 4”
Added: “--*Francisella tularensis* specifically *F. tularensis* subspecies *novocida* [aka *F. novocida*], strain Utah 112; *F. tularensis* subspecies *holartica* LVS; *F. tularensis* biovar *tularensis* strain ATCC 6223 (aka strain B38)”
Added footnote: “* For research involving high concentrations, BL3 practices should be considered (See [Appendix G-II-C-2](#). Special Practices (BL3)).”
- Page 40: Added “--*Yersinia pestis* specifically *pgm*⁽⁻⁾ strains (lacking the 102 kb pigmentation locus) and *lcr*⁽⁻⁾ strains (lacking the LCR plasmid)”
- Page 41: [Appendix B-II-D](#):
Added: “--Chikungunya vaccine strain 181/25”
“--Venezuelan equine encephalomyelitis vaccine strain TC-83” changed to:
“--Venezuelan equine encephalomyelitis vaccine strains TC-83 and V3526”
Added: “--Junin virus candid #1 vaccine strain”
Deleted “(Togaviruses)” from the Flavivirus group (also on page 43)
Added: “--Japanese encephalitis virus strain SA 14-14-2”
- Page 42: Corrected: “Papovaviruses” changed to “Papilloma viruses”
Changed: “--Vesicular stomatitis virus—laboratory adapted strains including VSV-Indiana, San Juan, and Glasgow” to: “--Vesicular stomatitis virus non exotic strains: VSV-Indiana 1 serotype strains (e.g. Glasgow, Mudd-Summers, Orsay, San Juan) and VSV-New Jersey serotype strains (e.g. Ogden, Hazelhurst)”
Corrected: “Rubivirus” as a genus name and “Rubella virus” as the type species
- Page 43: [Appendix B-III-D](#):
Added: “--Chikungunya virus (except those strains listed in [Appendix B-II-D Risk Group2 \(RG2\) – Viruses](#))”
Added: “Coronaviruses” and “--SARS-associated coronavirus (SARS-CoV)”
Added : “West Nile Virus (WNV)”
Added references to [Appendix B-II-D Risk Group2 \(RG2\) – Viruses](#) where applicable
- Page 44: [Appendix B-V](#)
Corrected: “Papovaviruses” changed to 1) “Papilloma viruses” (listing Bovine and Shope viruses) and 2) “Polyoma viruses” (listing Polyoma and SV40 viruses)

NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES (NIH GUIDELINES)

October 2011

DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

Visit the OBA Web site at:

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For current information on Guidelines, Protocols, Principal Investigators, Meetings,
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These *NIH Guidelines* supersede all earlier versions and shall be in effect until further notice.

FEDERAL REGISTER NOTICES

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SECTION I. SCOPE OF THE NIH GUIDELINES

Section I-A. Purpose

The purpose of the *NIH Guidelines* is to specify practices for constructing and handling: (i) recombinant deoxyribonucleic acid (DNA) molecules, and (ii) organisms and viruses containing recombinant DNA molecules.

Section I-A-1. Any recombinant DNA experiment, which according to the *NIH Guidelines* requires approval by NIH, must be submitted to NIH or to another Federal agency that has jurisdiction for review and approval. Once approvals, or other applicable clearances, have been obtained from a Federal agency other than NIH (whether the experiment is referred to that agency by NIH or sent directly there by the submitter), the experiment may proceed without the necessity for NIH review or approval. (See exception in [Section I-A-1-a](#) regarding requirement for human gene transfer protocol registration.)

Section I-A-1-a. For experiments involving the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into human research participants (human gene transfer), no research participant shall be enrolled (see definition of enrollment in [Section I-E-7](#)) until the RAC review process has been completed (see [Appendix M-I-B, RAC Review Requirements](#)); Institutional Biosafety Committee (IBC) approval (from the clinical trial site) has been obtained; Institutional Review Board approval has been obtained; and all applicable regulatory authorization(s) have been obtained.

For a clinical trial site that is added after the RAC review process, no research participant shall be enrolled (see definition of enrollment in [Section I-E-7](#)) at the clinical trial site until the following documentation has been submitted to NIH OBA: (1) IBC approval (from the clinical trial site); (2) Institutional Review Board approval; (3) Institutional Review Board-approved informed consent document; and (4) curriculum vitae of the principal investigator(s) (no more than two pages in biographical sketch format); and (5) NIH grant number(s) if applicable.

Section I-B. Definition of Recombinant DNA Molecules

In the context of the *NIH Guidelines*, recombinant DNA molecules are defined as either: (i) molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above.

Synthetic DNA segments which are likely to yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent) are considered as equivalent to their natural DNA counterpart. If the synthetic DNA segment is not expressed *in vivo* as a biologically active polynucleotide or polypeptide product, it is exempt from the *NIH Guidelines*.

Genomic DNA of plants and bacteria that have acquired a transposable element, even if the latter was donated from a recombinant vector no longer present, are not subject to the *NIH Guidelines* unless the transposon itself contains recombinant DNA.

Section I-C. General Applicability

Section I-C-1. The *NIH Guidelines* are applicable to:

Section I-C-1-a. All recombinant DNA research within the United States (U.S.) or its territories that is within the category of research described in either [Section I-C-1-a\(1\)](#) or [Section I-C-1-a\(2\)](#).

Section I-C-1-a(1). Research that is conducted at or sponsored by an institution that receives any support for recombinant DNA research from NIH, including research performed directly by NIH. An individual who receives support for research involving recombinant DNA must be associated with or sponsored by an institution that assumes the responsibilities assigned in the *NIH Guidelines*.

Section I-C-1-a-(2). Research that involves testing in humans of materials containing recombinant DNA developed with NIH funds, if the institution that developed those materials sponsors or participates in those projects. Participation includes research collaboration or contractual agreements, not mere provision of research materials.

Section I-C-1-b. All recombinant DNA research performed abroad that is within the category of research described in either Section I-C-1-b-(1) or Section I-C-1-b-(2).

Section I-C-1-b-(1). Research supported by NIH funds.

Section I-C-1-b-(2). Research that involves testing in humans of materials containing recombinant DNA developed with NIH funds, if the institution that developed those materials sponsors or participates in those projects. Participation includes research collaboration or contractual agreements, not mere provision of research materials.

Section I-C-1-b-(3). If the host country has established rules for the conduct of recombinant DNA research, then the research must be in compliance with those rules. If the host country does not have such rules, the proposed research must be reviewed and approved by an NIH-approved Institutional Biosafety Committee or equivalent review body and accepted in writing by an appropriate national governmental authority of the host country. The safety practices that are employed abroad must be reasonably consistent with the *NIH Guidelines*.

Section I-D. Compliance with the *NIH Guidelines*

As a condition for NIH funding of recombinant DNA research, institutions shall ensure that such research conducted at or sponsored by the institution, irrespective of the source of funding, shall comply with the *NIH Guidelines*.

Information concerning noncompliance with the *NIH Guidelines* may be brought forward by any person. It should be delivered to both NIH/OBA and the relevant institution. The institution, generally through the Institutional Biosafety Committee, shall take appropriate action. The institution shall forward a complete report of the incident recommending any further action to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985, 301-496-9838/301-496-9839 (fax) (for non-USPS mail, use zip code 20817).

In cases where NIH proposes to suspend, limit, or terminate financial assistance because of noncompliance with the *NIH Guidelines*, applicable DHHS and Public Health Service procedures shall govern.

The policies on compliance are as follows:

Section I-D-1. All NIH-funded projects involving recombinant DNA techniques must comply with the *NIH Guidelines*. Non-compliance may result in: (i) suspension, limitation, or termination of financial assistance for the noncompliant NIH-funded research project and of NIH funds for other recombinant DNA research at the institution, or (ii) a requirement for prior NIH approval of any or all recombinant DNA projects at the institution.

Section I-D-2. All non-NIH funded projects involving recombinant DNA techniques conducted at or sponsored by an institution that receives NIH funds for projects involving such techniques must comply with the *NIH Guidelines*. Noncompliance may result in: (i) suspension, limitation, or termination of NIH funds for recombinant DNA research at the institution, or (ii) a requirement for prior NIH approval of any or all recombinant DNA projects at the institution.

Section I-E. General Definitions

The following terms, which are used throughout the *NIH Guidelines*, are defined as follows:

Section I-E-1. An "institution" is any public or private entity (including Federal, state, and local government agencies).

Section I-E-2. An "Institutional Biosafety Committee" is a committee that: (i) meets the requirements for membership specified in [Section IV-B-2, Institutional Biosafety Committee \(IBC\)](#), and (ii) reviews, approves, and oversees projects in accordance with the responsibilities defined in [Section IV-B-2, Institutional Biosafety Committee \(IBC\)](#).

Section I-E-3. The "Office of Biotechnology Activities (OBA)" is the office within the NIH that is responsible for: (i) reviewing and coordinating all activities relating to the *NIH Guidelines*, and (ii) performing other duties as defined in [Section IV-C-3, Office of Biotechnology Activities \(OBA\)](#).

Section I-E-4. The "Recombinant DNA Advisory Committee" is the public advisory committee that advises the Department of Health and Human Services (DHHS) Secretary, the DHHS Assistant Secretary for Health, and the NIH Director concerning recombinant DNA research. The RAC shall be constituted as specified in [Section IV-C-2, Recombinant DNA Advisory Committee \(RAC\)](#).

Section I-E-5. The "NIH Director" is the Director of the National Institutes of Health, or any other officer or employee of NIH to whom authority has been delegated.

Section I-E-6. "Deliberate release" is defined as a planned introduction of recombinant DNA-containing microorganisms, plants, or animals into the environment.

Section I-E-7. "Enrollment" is the process of obtaining informed consent from a potential research participant, or a designated legal guardian of the participant, to undergo a test or procedure associated with the gene transfer experiment.

Section I-E-8. A "serious adverse event" is any event occurring at any dose that results in any of the following outcomes: death, a life-threatening event, in-patient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization also may be considered a serious adverse event when, upon the basis of appropriate medical judgment, they may jeopardize the human gene transfer research subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Section I-E-9. An adverse event is "associated with the use of a gene transfer product" when there is a reasonable possibility that the event may have been caused by the use of that product.

Section I-E-10. An "unexpected serious adverse event" is any serious adverse event for which the specificity or severity is not consistent with the risk information available in the current investigator's brochure.

SECTION II. SAFETY CONSIDERATIONS

Section II-A. Risk Assessment

Section II-A-1. Risk Groups

Risk assessment is ultimately a subjective process. The investigator must make an initial risk assessment based on the Risk Group (RG) of an agent (see [Appendix B, Classification of Human Etiologic Agents on the Basis of Hazard](#)). Agents are classified into four Risk Groups (RGs) according to their relative pathogenicity for healthy adult humans by the following criteria: (1) Risk Group 1 (RG1) agents are not associated with disease in healthy adult humans. (2) Risk Group 2 (RG2) agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available. (3) Risk Group 3 (RG3) agents are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available. (4) Risk Group 4 (RG4) agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available.

Section II-A-2. Criteria for Risk Groups

Classification of agents in [Appendix B, Classification of Human Etiologic Agents on the Basis of Hazard](#), is based on the potential effect of a biological agent on a healthy human adult and does not account for instances in which an individual may have increased susceptibility to such agents, e.g., preexisting diseases, medications, compromised immunity, pregnancy or breast feeding (which may increase exposure of infants to some agents).

Personnel may need periodic medical surveillance to ascertain fitness to perform certain activities; they may also need to be offered prophylactic vaccines and boosters (see [Section IV-B-1-f, Responsibilities of the Institution, General Information](#)).

Section II-A-3. Comprehensive Risk Assessment

In deciding on the appropriate containment for an experiment, the initial risk assessment from [Appendix B, Classification of Human Etiologic Agents on the Basis of Hazard](#), should be followed by a thorough consideration of the agent itself and how it is to be manipulated. Factors to be considered in determining the level of containment include agent factors such as: virulence, pathogenicity, infectious dose, environmental stability, route of spread, communicability, operations, quantity, availability of vaccine or treatment, and gene product effects such as toxicity, physiological activity, and allergenicity. Any strain that is known to be more hazardous than the parent (wild-type) strain should be considered for handling at a higher containment level. Certain attenuated strains or strains that have been demonstrated to have irreversibly lost known virulence factors may qualify for a reduction of the containment level compared to the Risk Group assigned to the parent strain (see [Appendix B Classification of Human Etiologic Agents on the Basis of Hazard](#) and [Section V-B, Footnotes and References of Sections I-IV](#)).

A final assessment of risk based on these considerations is then used to set the appropriate containment conditions for the experiment (see [Section II-B, Containment](#)). The containment level required may be equivalent to the Risk Group classification of the agent or it may be raised or lowered as a result of the above considerations. The Institutional Biosafety Committee must approve the risk assessment and the biosafety containment level for recombinant DNA experiments described in [Sections III-A, Experiments that Require Institutional Biosafety Committee Approval, RAC Review, and NIH Director Approval Before Initiation](#); [III-B, Experiments that Require NIH/OBA and Institutional Biosafety Committee Approval Before Initiation](#); [III-C, Experiments that Require Institutional Biosafety Committee and Institutional Review Board Approvals and NIH/OBA Registration Before Initiation](#); [III-D, Experiments that Require Institutional Biosafety Committee Approval Before Initiation](#).

Careful consideration should be given to the types of manipulation planned for some higher Risk Group agents. For example, the RG2 dengue viruses may be cultured under the Biosafety Level (BL) 2 containment (see [Section II-B](#)); however, when such agents are used for animal inoculation or transmission studies, a higher containment level is recommended. Similarly, RG3 agents such as Venezuelan equine encephalomyelitis and yellow fever viruses should be handled at a higher containment level for animal inoculation and transmission experiments.

Individuals working with human immunodeficiency virus (HIV), hepatitis B virus (HBV) or other bloodborne pathogens should consult the applicable [Occupational Safety and Health Administration \(OSHA\)](#) regulation, 29 CFR 1910.1030, and OSHA publication 3127 (1996 revised). BL2 containment is recommended for activities involving all blood-contaminated clinical specimens, body fluids, and tissues from all humans, or from HIV- or HBV-infected or inoculated laboratory animals. Activities such as the production of research-laboratory scale quantities of HIV or other bloodborne pathogens, manipulating concentrated virus preparations, or conducting procedures that may produce droplets or aerosols, are performed in a BL2 facility using the additional practices and containment equipment recommended for BL3. Activities involving industrial scale volumes or preparations of concentrated HIV are conducted in a BL3 facility, or BL3 Large Scale if appropriate, using BL3 practices and containment equipment.

Exotic plant pathogens and animal pathogens of domestic livestock and poultry are restricted and may require special laboratory design, operation and containment features not addressed in *Biosafety in Microbiological and Biomedical Laboratories* (see [Section V-C, Footnotes and References of Sections I through IV](#)). For information regarding the importation, possession, or use of these agents see [Sections V-G and V-H, Footnotes and References of Sections I through IV](#).

Section II-B. Containment

Effective biological safety programs have been operative in a variety of laboratories for many years. Considerable information already exists about the design of physical containment facilities and selection of laboratory procedures applicable to organisms carrying recombinant DNA (see [Section V-B, Footnotes and References of Sections I-IV](#)). The existing programs rely upon mechanisms that can be divided into two categories: (i) a set of standard practices that are generally used in microbiological laboratories; and (ii) special procedures, equipment, and laboratory installations that provide physical barriers that are applied in varying degrees according to the estimated biohazard. Four biosafety levels are described in [Appendix G, Physical Containment](#). These biosafety levels consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities appropriate for the operations performed and are based on the potential hazards imposed by the agents used and for the laboratory function and activity. Biosafety Level 4 provides the most stringent containment conditions, Biosafety Level 1 the least stringent.

Experiments involving recombinant DNA lend themselves to a third containment mechanism, namely, the application of highly specific biological barriers. Natural barriers exist that limit either: (i) the infectivity of a vector or vehicle (plasmid or virus) for specific hosts, or (ii) its dissemination and survival in the environment. Vectors, which provide the means for recombinant DNA and/or host cell replication, can be genetically designed to decrease, by many orders of magnitude, the probability of dissemination of recombinant DNA outside the laboratory (see [Appendix I, Biological Containment](#)).

Since these three means of containment are complementary, different levels of containment can be established that apply various combinations of the physical and biological barriers along with a constant use of standard practices. Categories of containment are considered separately in order that such combinations can be conveniently expressed in the *NIH Guidelines*.

Physical containment conditions within laboratories, described in [Appendix G, Physical Containment](#), may not always be appropriate for all organisms because of their physical size, the number of organisms needed for an experiment, or the particular growth requirements of the organism. Likewise, biological containment for microorganisms described in [Appendix I, Biological Containment](#), may not be appropriate for all organisms, particularly higher eukaryotic organisms. However, significant information exists about the design of research facilities and experimental procedures that are applicable to organisms containing recombinant DNA that is either integrated into the genome or into microorganisms associated with the higher organism as a symbiont, pathogen, or other relationship. This information describes facilities for physical containment of organisms used in non-traditional laboratory settings and special practices for limiting or excluding the unwanted establishment, transfer of genetic information, and dissemination of organisms beyond the intended location, based on both physical and biological containment principles. Research conducted in accordance with these conditions effectively confines the organism.

For research involving plants, four biosafety levels (BL1-P through BL4-P) are described in [Appendix P, Physical and Biological Containment for Recombinant DNA Research Involving Plants](#). BL1-P is designed to provide a moderate level of containment for experiments for which there is convincing biological evidence that precludes the possibility of survival, transfer, or dissemination of recombinant DNA into the environment, or in which there is no recognizable and predictable risk to the environment in the event of accidental release. BL2-P is designed to provide a greater level of containment for experiments involving plants and certain associated organisms in which there is a recognized possibility of survival, transmission, or dissemination of recombinant DNA containing organisms, but the consequence of such an inadvertent release has a predictably minimal biological impact. BL3-P and BL4-P describe additional containment conditions for research with plants and certain pathogens and other organisms that require special containment because of their recognized potential for significant detrimental impact on managed or natural ecosystems. BL1-P relies upon accepted scientific practices for conducting research in most ordinary greenhouse or growth chamber facilities and incorporates accepted procedures for good pest control and cultural practices. BL1-P facilities and procedures provide a modified and protected environment for the propagation of plants and microorganisms associated with the plants and a degree of containment that adequately controls the potential for release of biologically viable plants, plant parts, and microorganisms associated with them. BL2-P and BL3-P rely upon accepted scientific practices for conducting research in greenhouses with organisms infecting or infesting plants in a manner that minimizes or prevents inadvertent contamination of plants within or surrounding the greenhouse. BL4-P describes facilities and practices known to provide containment of certain exotic plant pathogens.

For research involving animals, which are of a size or have growth requirements that preclude the use of conventional primary containment systems used for small laboratory animals, four biosafety levels (BL1-N through BL4-N) are described in [Appendix Q, Physical and Biological Containment for Recombinant DNA Research Involving Animals](#). BL1-N describes containment for animals that have been modified by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant DNA-modified microorganisms and is designed to eliminate the possibility of sexual transmission of the modified genome or transmission of recombinant DNA-derived viruses known to be transmitted from animal parent to offspring only by sexual reproduction. Procedures, practices, and facilities follow classical methods of avoiding genetic exchange between animals. BL2-N describes containment which is used for transgenic animals associated with recombinant DNA-derived organisms and is designed to eliminate the possibility of vertical or horizontal transmission. Procedures, practices, and facilities follow classical methods of avoiding genetic exchange between animals or controlling arthropod transmission. BL3-N and BL4-N describe higher levels of containment for research with certain transgenic animals involving agents which pose recognized hazard.

In constructing the *NIH Guidelines*, it was necessary to define boundary conditions for the different levels of physical and biological containment and for the classes of experiments to which they apply. These definitions do not take into account all existing and anticipated information on special procedures that will allow particular experiments to be conducted under different conditions than indicated here without affecting risk. Individual investigators and Institutional Biosafety Committees are urged to devise simple and more effective containment procedures and to submit recommended changes in the *NIH Guidelines* to permit the use of these procedures.

SECTION III. EXPERIMENTS COVERED BY THE NIH GUIDELINES

This section describes six categories of experiments involving recombinant DNA: (i) those that require Institutional Biosafety Committee (IBC) approval, RAC review, and NIH Director approval before initiation (see [Section III-A](#)), (ii) those that require NIH/OBA and Institutional Biosafety Committee approval before initiation (see [Section III-B](#)), (iii) those that require Institutional Biosafety Committee and Institutional Review Board approvals and RAC review before research participant enrollment (see [Section III-C](#)), (iv) those that require Institutional Biosafety Committee approval before initiation (see [Section III-D](#)), (v) those that require Institutional Biosafety Committee notification simultaneous with initiation (see [Section III-E](#)), and (vi) those that are exempt from the *NIH Guidelines* (see [Section III-F](#)).

Note: *If an experiment falls into Sections III-A, III-B, or III-C and one of the other sections, the rules pertaining to Sections III-A, III-B, or III-C shall be followed. If an experiment falls into Section III-F and into either Sections III-D or III-E as well, the experiment is considered exempt from the NIH Guidelines.*

Any change in containment level, which is different from those specified in the *NIH Guidelines*, may not be initiated without the express approval of NIH/OBA (see [Section IV-C-1-b-\(2\)](#) and its subsections, *Minor Actions*).

Section III-A. Experiments that Require Institutional Biosafety Committee Approval, RAC Review, and NIH Director Approval Before Initiation (See [Section IV-C-1-b-\(1\)](#), Major Actions).

Section III-A-1. Major Actions under the NIH Guidelines

Experiments considered as *Major Actions* under the *NIH Guidelines* cannot be initiated without submission of relevant information on the proposed experiment to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax), the publication of the proposal in the *Federal Register* for 15 days of comment, review by RAC, and specific approval by NIH. The containment conditions or stipulation requirements for such experiments will be recommended by RAC and set by NIH at the time of approval. Such experiments require Institutional Biosafety Committee approval before initiation. Specific experiments already approved are included in [Appendix D, Major Actions Taken under the NIH Guidelines](#), which may be obtained from the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

Section III-A-1-a. The deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally (see [Section V-B](#), *Footnotes and References of Sections I-IV*), if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture, will be reviewed by RAC.

Section III-B. Experiments That Require NIH/OBA and Institutional Biosafety Committee Approval Before Initiation

Experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH/OBA. The containment conditions for such experiments will be determined by NIH/OBA in consultation with *ad hoc* experts. Such experiments require Institutional Biosafety Committee approval before initiation (see [Section IV-B-2-b-\(1\)](#), *Institutional Biosafety Committee*).

Section III-B-1. Experiments Involving the Cloning of Toxin Molecules with LD₅₀ of Less than 100 Nanograms per Kilogram Body Weight

Deliberate formation of recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD₅₀ of less than 100 nanograms per kilogram body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, and *Shigella dysenteriae* neurotoxin). Specific approval has been given for the cloning in *Escherichia coli* K-12 of DNA containing genes coding for the biosynthesis of toxic molecules which are lethal to vertebrates at 100 nanograms to 100 micrograms per kilogram body weight. Specific experiments already approved under this section may be obtained from the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

Section III-C. Experiments that Require Institutional Biosafety Committee and Institutional Review Board Approvals and RAC Review Before Research Participant Enrollment

Section III-C-1. Experiments Involving the Deliberate Transfer of Recombinant DNA, or DNA or RNA Derived from Recombinant DNA, into One or More Human Research Participants

For an experiment involving the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into human research participants (human gene transfer), no research participant shall be enrolled (see definition of enrollment in [Section I-E-7](#)) until the RAC review process has been completed (see [Appendix M-I-B](#), *RAC Review Requirements*).

In its evaluation of human gene transfer proposals, the RAC will consider whether a proposed human gene transfer experiment presents characteristics that warrant public RAC review and discussion (See [Appendix M-I-B-2](#)). The process of public RAC review and discussion is intended to foster the safe and ethical conduct of human gene transfer experiments. Public review and discussion of a human gene transfer experiment (and access to relevant information) also serves to inform the public about the technical aspects of the proposal, meaning and significance of the research, and any significant safety, social, and ethical implications of the research.

Public RAC review and discussion of a human gene transfer experiment may be: (1) initiated by the NIH Director; or (2) initiated by the NIH OBA Director following a recommendation to NIH OBA by: (a) three or more RAC members; or (b) a Federal agency other than NIH. After a human gene transfer experiment is reviewed by the RAC at a regularly scheduled meeting, NIH OBA will send a letter, unless NIH OBA determines that there are exceptional circumstances, within 10 working days to the NIH Director, the Principal Investigator, the sponsoring institution, and other DHHS components, as appropriate, summarizing the RAC recommendations.

For a clinical trial site that is added after the RAC review process, no research participant shall be enrolled (see definition of enrollment in [Section I-E-7](#)) at the clinical trial site until the following documentation has been submitted to NIH OBA: (1) Institutional Biosafety Committee approval (from the clinical trial site); (2) Institutional Review Board approval; (3) Institutional Review Board-approved informed consent document; (4) curriculum vitae of the principal investigator(s) (no more than two pages in biographical sketch format); and (5) NIH grant number(s) if applicable.

In order to maintain public access to information regarding human gene transfer protocols (including protocols that are not publicly reviewed by the RAC), NIH OBA will maintain the documentation described in Appendices M-I through M-V. The information provided in response to [Appendix M](#) should not contain any confidential commercial information or trade secrets, enabling all aspects of RAC review to be open to the public.

Note: For specific directives concerning the use of retroviral vectors for gene delivery, consult [Appendix B-V-1, Murine Retroviral Vectors](#).

Section III-D. Experiments that Require Institutional Biosafety Committee Approval Before Initiation

Prior to the initiation of an experiment that falls into this category, the Principal Investigator must submit a registration document to the Institutional Biosafety Committee which contains the following information: (i) the source(s) of DNA; (ii) the nature of the inserted DNA sequences; (iii) the host(s) and vector(s) to be used; (iv) if an attempt will be made to obtain expression of a foreign gene, and if so, indicate the protein that will be produced; and (v) the containment conditions that will be implemented as specified in the *NIH Guidelines*. For experiments in this category, the registration document shall be dated, signed by the Principal Investigator, and filed with the Institutional Biosafety Committee. The Institutional Biosafety Committee shall review and approve all experiments in this category prior to their initiation. Requests to decrease the level of containment specified for experiments in this category will be considered by NIH (see [Section IV-C-1-b-\(2\)-\(c\), Minor Actions](#)).

Section III-D-1. Experiments Using Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems (See [Section II-A, Risk Assessment](#))

Section III-D-1-a. Experiments involving the introduction of recombinant DNA into Risk Group 2 agents will usually be conducted at Biosafety Level (BL) 2 containment. Experiments with such agents will usually be conducted with whole animals at BL2 or BL2-N (Animals) containment.

Section III-D-1-b. Experiments involving the introduction of recombinant DNA into Risk Group 3 agents will usually be conducted at BL3 containment. Experiments with such agents will usually be conducted with whole animals at BL3 or BL3-N containment.

Section III-D-1-c. Experiments involving the introduction of recombinant DNA into Risk Group 4 agents shall be conducted at BL4 containment. Experiments with such agents shall be conducted with whole animals at BL4 or BL4-N containment.

Section III-D-1-d. Containment conditions for experiments involving the introduction of recombinant DNA into restricted agents shall be set on a case-by-case basis following NIH/OBA review. A U.S. Department of Agriculture - [Animal and Plant Health Inspection Service](#) (USDA/APHIS) permit is required for work with plant or animal pathogens (see [Section V-G and V-M, Footnotes and References of Sections I-IV](#)). Experiments with such agents shall be conducted with whole animals at BL4 or BL4-N containment.

Section III-D-2. Experiments in Which DNA From Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents is Cloned into Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems

Section III-D-2-a. Experiments in which DNA from Risk Group 2 or Risk Group 3 agents (see [Section II-A, Risk Assessment](#)) is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment. Experiments in which DNA from Risk Group 4 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment after demonstration that only a totally and irreversibly defective fraction of the agent's genome is present in a given recombinant. In the absence of such a demonstration, BL4 containment shall be used. The Institutional Biosafety Committee may approve the specific lowering of containment for particular experiments to BL1. Many experiments in this category are exempt from the *NIH Guidelines* (see [Section III-F, Exempt Experiments](#)). Experiments involving the formation of recombinant DNA for certain genes coding for molecules toxic for vertebrates require NIH/OBA approval (see [Section III-B-1, Experiments Involving the Cloning of Toxin Molecules with LD₅₀ of Less than 100 Nanograms Per Kilogram Body Weight](#)) or shall be conducted under NIH specified conditions as described in [Appendix F, Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates](#).

Section III-D-2-b. Containment conditions for experiments in which DNA from restricted agents is transferred into nonpathogenic prokaryotes or lower eukaryotes shall be determined by NIH/OBA following a case-by-case review (see [Section V-L, Footnotes and References of Sections I-IV](#)). A U.S. Department of Agriculture permit is required for work with plant or animal pathogens (see [Section V-G, Footnotes and References of Sections I-IV](#)).

Section III-D-3. Experiments Involving the Use of Infectious DNA or RNA Viruses or Defective DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems

Caution: Special care should be used in the evaluation of containment levels for experiments which are likely to either enhance the pathogenicity (e.g., insertion of a host oncogene) or to extend the host range (e.g., introduction of novel control elements) of viral vectors under conditions that permit a productive infection. In such cases, serious consideration should be given to increasing physical containment by at least one level.

Note: Recombinant DNA or RNA molecules derived therefrom, which contain less than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family (see [Section V-J, Footnotes and References of Sections I-IV](#)) being considered identical (see [Section V-K, Footnotes and References of Sections I-IV](#)), are considered defective and may be used in the absence of helper under the conditions specified in [Section III-E-1, Experiments Involving the Formation of Recombinant DNA Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus](#).

Section III-D-3-a. Experiments involving the use of infectious or defective Risk Group 2 viruses (see [Appendix B-II, Risk Group 2 Agents](#)) in the presence of helper virus may be conducted at BL2.

Section III-D-3-b. Experiments involving the use of infectious or defective Risk Group 3 viruses (see [Appendix B-III-D, Risk Group 3 \(RG3\) - Viruses and Prions](#)) in the presence of helper virus may be conducted at BL3.

Section III-D-3-c. Experiments involving the use of infectious or defective Risk Group 4 viruses (see [Appendix B-IV-D, Risk Group 4 \(RG4\) - Viral Agents](#)) in the presence of helper virus may be conducted at BL4.

Section III-D-3-d. Experiments involving the use of infectious or defective restricted poxviruses (see [Sections V-A and V-L, Footnotes and References of Sections I-IV](#)) in the presence of helper virus shall be determined on a case-by-case basis following NIH/OBA review. A U.S. Department of Agriculture permit is required for work with plant or animal pathogens (see [Section V-G, Footnotes and References of Sections I-IV](#)).

Section III-D-3-e. Experiments involving the use of infectious or defective viruses in the presence of helper virus which are not covered in [Sections III-D-3-a](#) through [III-D-3-d](#) may be conducted at BL1.

Section III-D-4. Experiments Involving Whole Animals

This section covers experiments involving whole animals in which the animal's genome has been altered by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant DNA-modified microorganisms tested on whole animals. For the latter, other than viruses which are only vertically transmitted, the experiments may *not* be conducted at BL1-N containment. A minimum containment of BL2 or BL2-N is required.

Caution - Special care should be used in the evaluation of containment conditions for some experiments with transgenic animals. For example, such experiments might lead to the creation of novel mechanisms or increased transmission of a recombinant pathogen or production of undesirable traits in the host animal. In such cases, serious consideration should be given to increasing the containment conditions.

Section III-D-4-a. Recombinant DNA, or DNA or RNA molecules derived therefrom, from any source except for greater than two-thirds of eukaryotic viral genome may be transferred to any non-human vertebrate or any invertebrate organism and propagated under conditions of physical containment comparable to BL1 or BL1-N and appropriate to the organism under study (see [Section V-B, Footnotes and References of Sections I-IV](#)). Animals that contain sequences from viral vectors, which do not lead to transmissible infection either directly or indirectly as a result of complementation or recombination in animals, may be propagated under conditions of physical containment comparable to BL1 or BL1-N and appropriate to the organism under study. Experiments involving the introduction of other sequences from eukaryotic viral genomes into animals are covered under

Section III-D-4-b, Experiments Involving Whole Animals. For experiments involving recombinant DNA-modified Risk Groups 2, 3, 4, or restricted organisms, see [Sections V-A, V-G, and V-L, Footnotes and References of Sections I-IV](#). It is important that the investigator demonstrate that the fraction of the viral genome being utilized does not lead to productive infection. A U.S. Department of Agriculture permit is required for work with plant or animal pathogens (see [Section V-G, Footnotes and References of Sections I-IV](#)).

Section III-D-4-b. For experiments involving recombinant DNA, or DNA or RNA derived therefrom, involving whole animals, including transgenic animals, and not covered by [Sections III-D-1, Experiments Using Human or Animal Pathogens \(Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems, or III-D-4-a, Experiments Involving Whole Animals](#), the appropriate containment shall be determined by the Institutional Biosafety Committee.

Section III-D-4-c. Exceptions under [Section III-D-4, Experiments Involving Whole Animals](#)

Section III-D-4-c-(1). Experiments involving the generation of transgenic rodents that require BL1 containment are described under [Section III-E-3, Experiments Involving Transgenic Rodents](#).

Section III-D-4-c-(2). The purchase or transfer of transgenic rodents is exempt from the *NIH Guidelines* under [Section III-F, Exempt Experiments](#) (see [Appendix C-VI, The Purchase or Transfer of Transgenic Rodents](#)).

Section III-D-5. Experiments Involving Whole Plants

Experiments to genetically engineer plants by recombinant DNA methods, to use such plants for other experimental purposes (e.g., response to stress), to propagate such plants, or to use plants together with microorganisms or insects containing recombinant DNA, may be conducted under the containment conditions described in [Sections III-D-5-a through III-D-5-e](#). If experiments involving whole plants are not described in [Section III-D-5](#) and do not fall under [Sections III-A, III-B, III-D or III-F](#), they are included in [Section III-E](#).

NOTE - For recombinant DNA experiments falling under [Sections III-D-5-a through III-D-5-d](#), physical containment requirements may be reduced to the next lower level by appropriate biological containment practices, such as conducting experiments on a virus with an obligate insect vector in the absence of that vector or using a genetically attenuated strain.

Section III-D-5-a. BL3-P (Plants) or BL2-P + biological containment is recommended for experiments involving most exotic (see [Section V-M, Footnotes and References of Sections I-IV](#)) infectious agents with recognized potential for serious detrimental impact on managed or natural ecosystems when recombinant DNA techniques are associated with whole plants.

Section III-D-5-b. BL3-P or BL2-P + biological containment is recommended for experiments involving plants containing cloned genomes of readily transmissible exotic (see [Section V-M, Footnotes and References of Sections I-IV](#)) infectious agents with recognized potential for serious detrimental effects on managed or natural ecosystems in which there exists the possibility of reconstituting the complete and functional genome of the infectious agent by genomic complementation *in planta*.

Section III-D-5-c. BL4-P containment is recommended for experiments with a small number of readily transmissible exotic (see [Section V-M, Footnotes and References of Sections I-IV](#)) infectious agents, such as the soybean rust fungus (*Phakospora pachyrhizi*) and maize streak or other viruses in the presence of their specific arthropod vectors, that have the potential of being serious pathogens of major U.S. crops.

Section III-D-5-d. BL3-P containment is recommended for experiments involving sequences encoding potent vertebrate toxins introduced into plants or associated organisms. Recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD₅₀ of <100 nanograms per kilogram body weight fall under [Section III-B-1, Experiments Involving the Cloning of Toxin Molecules with LD₅₀ of Less than 100 Nanograms Per Kilogram Body Weight](#), and require NIH/OBA and Institutional Biosafety Committee approval before initiation.

Section III-D-5-e. BL3-P or BL2-P + biological containment is recommended for experiments with microbial pathogens of insects or small animals associated with plants if the recombinant DNA-modified organism has a recognized potential for serious detrimental impact on managed or natural ecosystems.

Section III-D-6. Experiments Involving More than 10 Liters of Culture

The appropriate containment will be decided by the Institutional Biosafety Committee. Where appropriate, [Appendix K, Physical Containment for Large Scale Uses of Organisms Containing Recombinant DNA Molecules](#), shall be used. [Appendix K](#) describes containment conditions Good Large Scale Practice through BL3-Large Scale.

Section III-D-7. Experiments Involving Influenza Viruses

Experiments with influenza viruses generated by recombinant methods (e.g., generation by reverse genetics of chimeric viruses with reassorted segments, introduction of specific mutations) shall be conducted at the biosafety level containment corresponding to the risk group of the virus that was the source of the majority of segments in the recombinant virus (e.g., experiments with viruses containing a majority of segments from a RG3 virus shall be conducted at BL3). Experiments with influenza viruses containing genes or segments from 1918-1919 H1N1 (1918 H1N1), human H2N2 (1957-1968) and highly pathogenic avian influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1) shall be conducted at BL3 enhanced containment (see [Appendix G-II-C-5, Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses](#)) unless indicated below.

Section III-D-7-a. Human H2N2 (1957-1968). Experiments with influenza viruses containing the H2 hemagglutinin (HA) segment shall be conducted at BL3 enhanced (see [Appendix G-II-C-5, Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses](#)). Experiments with the H2 HA gene in cold-adapted, live attenuated vaccine strains (e.g., A/Ann Arbor/6/60 H2N2) may be conducted at BL2 containment provided segments with mutations conferring temperature sensitivity and attenuation are not altered in the recombinant virus. Experiments with Risk Group 2 influenza viruses containing genes from human H2N2 other than the HA gene can be worked on at BL2.

Section III-D-7-b. Highly Pathogenic Avian Influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1). Experiments involving influenza viruses containing a majority of genes and/or segments from a HPAI H5N1 influenza virus shall be conducted at BL3 enhanced containment, (see [Appendix G-II-C-5, Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses](#)). Experiments involving influenza viruses containing a minority of genes and/or segments from a HPAI H5N1 influenza virus shall be conducted at BL3 enhanced unless a risk assessment performed by the IBC determines that they can be conducted safely at biosafety level 2 and after they have been excluded pursuant to [9 CFR 121.3\(e\)](#). OBA is available to IBCs to provide consultation with the RAC and influenza virus experts when risk assessments are being made to determine the appropriate biocontainment for experiments with influenza viruses containing a minority of gene/segments from HPAI H5N1. Such experiments may be performed at BL3 enhanced containment or containment may be lowered to biosafety level 2, the level of containment for most research with other influenza viruses. ([USDA/APHIS](#) regulations and decisions on lowering containment also apply.) In deciding to lower containment, the IBC should consider whether, in at least two animal models (e.g., ferret, mouse, Syrian golden hamster, cotton rat, non-human primates), there is evidence that the resulting influenza virus shows reduced replication and virulence compared to the parental RG3 virus at relevant doses. This should be determined by measuring biological indices appropriate for the specific animal model (e.g., severe weight loss, elevated temperature, mortality or neurological symptoms).

Section III-D-7-c. 1918 H1N1. Experiments involving influenza viruses containing any gene or segment from 1918 H1N1 shall be conducted at BL3 enhanced containment (see [Appendix G-II-C-5, Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses](#)).

Section III-D-7-d. Antiviral Susceptibility and Containment. The availability of antiviral drugs as preventive and therapeutic measures is an important safeguard for experiments with 1918 H1N1, HPAI H5N1, and human H2N2 (1957-1968). If an influenza virus containing genes from one of these viruses is resistant to both classes of current antiviral agents, adamantanes and neuraminidase inhibitors, higher containment may be required based on the risk assessment considering transmissibility to humans, virulence, pandemic potential, alternative antiviral agents if available, etc.

Experiments with 1918 H1N1, human H2N2 (1957-1968) or HPAI H5N1 that are designed to create resistance to neuraminidase inhibitors or other effective antiviral agents (including investigational antiviral agents being developed for influenza) would be subject to [Section III-A-1](#) (Major Actions) and require RAC review and NIH Director approval. As per [Section I-A-1](#) of the *NIH Guidelines*, if the agent is a Select Agent, the NIH will defer to the appropriate Federal agency (HHS or USDA Select Agent Divisions) on such experiments.

Section III-E. Experiments that Require Institutional Biosafety Committee Notice Simultaneous with Initiation

Experiments not included in Sections [III-A](#), [III-B](#), [III-C](#), [III-D](#), [III-F](#), and their subsections are considered in [Section III-E](#). All such experiments may be conducted at BL1 containment. For experiments in this category, a registration document (see [Section III-D](#), *Experiments that Require Institutional Biosafety Committee Approval Before Initiation*) shall be dated and signed by the investigator and filed with the local Institutional Biosafety Committee at the time the experiment is initiated. The Institutional Biosafety Committee reviews and approves all such proposals, but Institutional Biosafety Committee review and approval prior to initiation of the experiment is not required (see [Section IV-A](#), *Policy*). For example, experiments in which all components derived from non-pathogenic prokaryotes and non-pathogenic lower eukaryotes fall under [Section III-E](#) and may be conducted at BL1 containment.

Section III-E-1. Experiments Involving the Formation of Recombinant DNA Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus

Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family being considered identical [see [Section V-J](#), *Footnotes and References of Sections I-IV*]) may be propagated and maintained in cells in tissue culture using BL1 containment. For such experiments, it must be demonstrated that the cells lack helper virus for the specific Families of defective viruses being used. If helper virus is present, procedures specified under [Section III-D-3](#), *Experiments Involving the Use of Infectious Animal or Plant DNA or RNA Viruses or Defective Animal or Plant DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems*, should be used. The DNA may contain fragments of the genome of viruses from more than one Family but each fragment shall be less than two-thirds of a genome.

Section III-E-2. Experiments Involving Whole Plants

This section covers experiments involving recombinant DNA-modified whole plants, and/or experiments involving recombinant DNA-modified organisms associated with whole plants, except those that fall under [Section III-A](#), [III-B](#), [III-D](#), or [III-F](#). It should be emphasized that knowledge of the organisms and judgment based on accepted scientific practices should be used in all cases in selecting the appropriate level of containment. For example, if the genetic modification has the objective of increasing pathogenicity or converting a non-pathogenic organism into a pathogen, then a higher level of containment may be appropriate depending on the organism, its mode of dissemination, and its target organisms. By contrast, a lower level of containment may be appropriate for small animals associated with many types of recombinant DNA-modified plants.

Section III-E-2-a. BL1-P is recommended for all experiments with recombinant DNA-containing plants and plant-associated microorganisms not covered in [Section III-E-2-b](#) or other sections of the *NIH Guidelines*. Examples of such experiments are those involving recombinant DNA-modified plants that are not noxious weeds or that cannot interbreed with noxious weeds in the immediate geographic area, and experiments involving whole plants and recombinant DNA-modified non-exotic (see [Section V-M](#), *Footnotes and References of Sections I-IV*) microorganisms that have no recognized potential for rapid and widespread dissemination or for serious detrimental impact on managed or natural ecosystems (e.g., *Rhizobium* spp. and *Agrobacterium* spp.).

Section III-E-2-b. BL2-P or BL1-P + biological containment is recommended for the following experiments:

Section III-E-2-b-(1). Plants modified by recombinant DNA that are noxious weeds or can interbreed with noxious weeds in the immediate geographic area.

Section III-E-2-b-(2). Plants in which the introduced DNA represents the complete genome of a non-exotic infectious agent (see [Section V-M](#), *Footnotes and References of Sections I-IV*).

Section III-E-2-b-(3). Plants associated with recombinant DNA-modified non-exotic microorganisms that have a recognized potential for serious detrimental impact on managed or natural ecosystems (see [Section V-M](#), *Footnotes and References of Sections I-IV*).

Section III-E-2-b-(4). Plants associated with recombinant DNA-modified exotic microorganisms that have no recognized potential for serious detrimental impact on managed or natural ecosystems (see [Section V-M](#), *Footnotes and References of Sections I-IV*).

Section III-E-2-b-(5). Experiments with recombinant DNA-modified arthropods or small animals associated with plants, or with arthropods or small animals with recombinant DNA-modified microorganisms associated with them if the recombinant DNA-modified microorganisms have no recognized potential for serious detrimental impact on managed or natural ecosystems (see [Section V-M](#), *Footnotes and References of Sections I-IV*).

Section III-E-3. Experiments Involving Transgenic Rodents

This section covers experiments involving the generation of rodents in which the animal's genome has been altered by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic rodents). Only experiments that require BL1 containment are covered under this section; experiments that require BL2, BL3, or BL4 containment are covered under [Section III-D-4, Experiments Involving Whole Animals](#).

Section III-E-3-a. Experiments involving the breeding of certain BL1 transgenic rodents are exempt under [Section III-F, Exempt Experiments](#) (See [Appendix C-VII, Generation of BL1 Transgenic Rodents via Breeding](#)).

Section III-F. Exempt Experiments

The following recombinant DNA molecules are exempt from the *NIH Guidelines* and registration with the Institutional Biosafety Committee is not required:

Section III-F-1. Those that are not in organisms or viruses.

Section III-F-2. Those that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent.

Section III-F-3. Those that consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well established physiological means.

Section III-F-4. Those that consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).

Section III-F-5. Those that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the NIH Director with advice of the RAC after appropriate notice and opportunity for public comment (see [Section IV-C-1-b-\(1\)-\(c\), Major Actions](#)). See [Appendices A-I through A-VI, Exemptions Under Section III-F-5--Sublists of Natural Exchangers](#), for a list of natural exchangers that are exempt from the *NIH Guidelines*.

Section III-F-6. Those that do not present a significant risk to health or the environment (see [Section IV-C-1-b-\(1\)-\(c\), Major Actions](#)), as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment. See [Appendix C, Exemptions under Section III-F-6](#) for other classes of experiments which are exempt from the *NIH Guidelines*.

SECTION IV. ROLES AND RESPONSIBILITIES

Section IV-A. Policy

The safe conduct of experiments involving recombinant DNA depends on the individual conducting such activities. The *NIH Guidelines* cannot anticipate every possible situation. Motivation and good judgment are the key essentials to protection of health and the environment. The *NIH Guidelines* are intended to assist the institution, Institutional Biosafety Committee, Biological Safety Officer, and the Principal Investigator in determining safeguards that should be implemented. The *NIH Guidelines* will never be complete or final since all conceivable experiments involving recombinant DNA cannot be foreseen. Therefore, *it is the responsibility of the institution and those associated with it to adhere to the intent of the NIH Guidelines as well as to their specifics*. Each institution (and the Institutional Biosafety Committee acting on its behalf) is responsible for ensuring that all recombinant DNA research conducted at or sponsored by that institution is conducted in compliance with the *NIH Guidelines*. General recognition of institutional authority and responsibility properly establishes accountability for safe conduct of the research at the local level. The following roles and responsibilities constitute an administrative framework in which safety is an essential and integral part of research involving recombinant DNA molecules. Further clarifications and interpretations of roles and responsibilities will be issued by NIH as necessary.

Section IV-B. Responsibilities of the Institution

Section IV-B-1. General Information

Each institution conducting or sponsoring recombinant DNA research which is covered by the *NIH Guidelines* is responsible for ensuring that the research is conducted in full conformity with the provisions of the *NIH Guidelines*. In order to fulfill this responsibility, the institution shall:

Section IV-B-1-a. Establish and implement policies that provide for the safe conduct of recombinant DNA research and that ensure compliance with the *NIH Guidelines*. As part of its general responsibilities for implementing the *NIH Guidelines*, the institution may establish additional procedures, as deemed necessary, to govern the institution and its components in the discharge of its responsibilities under the *NIH Guidelines*. Such procedures may include: (i) statements formulated by the institution for the general implementation of the *NIH Guidelines*, and (ii) any additional precautionary steps the institution deems appropriate.

Section IV-B-1-b. Establish an Institutional Biosafety Committee that meets the requirements set forth in Section IV-B-2-a and carries out the functions detailed in [Section IV-B-2-b](#).

Section IV-B-1-c. Appoint a Biological Safety Officer (who is also a member of the Institutional Biosafety Committee) if the institution: (i) conducts recombinant DNA research at Biosafety Level (BL) 3 or BL4, or (ii) engages in large-scale (greater than 10 liters) research. The Biological Safety Officer carries out the duties specified in [Section IV-B-3](#).

Section IV-B-1-d. Appoint at least one individual with expertise in plant, plant pathogen, or plant pest containment principles (who is a member of the Institutional Biosafety Committee) if the institution conducts recombinant DNA research that requires Institutional Biosafety Committee approval in accordance with [Appendix P](#), *Physical and Biological Containment for Recombinant DNA Research Involving Plants*.

Section IV-B-1-e. Appoint at least one individual with expertise in animal containment principles (who is a member of the Institutional Biosafety Committee) if the institution conducts recombinant DNA research that requires Institutional Biosafety Committee approval in accordance with [Appendix Q](#), *Physical and Biological Containment for Recombinant DNA Research Involving Animals*.

Section IV-B-1-f. Ensure that when the institution participates in or sponsors recombinant DNA research involving human subjects: (i) the Institutional Biosafety Committee has adequate expertise and training (using *ad hoc* consultants as deemed necessary), (ii) all aspects of [Appendix M](#) have been appropriately addressed by the Principal Investigator; and (iii) no research participant shall be enrolled (see definition of enrollment in [Section I-E-7](#)) in a human gene transfer experiment until the RAC review process has been completed (see [Appendix M-I-B](#), *RAC Review Requirements*), Institutional Biosafety Committee approval has been obtained,

Institutional Review Board approval has been obtained, and all applicable regulatory authorizations have been obtained. Institutional Biosafety Committee approval must be obtained from each institution at which recombinant DNA material will be administered to human subjects (as opposed to each institution involved in the production of vectors for human application and each institution at which there is *ex vivo* transduction of recombinant DNA material into target cells for human application).

Section IV-B-1-g. Assist and ensure compliance with the *NIH Guidelines* by Principal Investigators conducting research at the institution as specified in [Section IV-B-7](#).

Section IV-B-1-h. Ensure appropriate training for the Institutional Biosafety Committee Chair and members, Biological Safety Officer and other containment experts (when applicable), Principal Investigators, and laboratory staff regarding laboratory safety and implementation of the *NIH Guidelines*. The Institutional Biosafety Committee Chair is responsible for ensuring that Institutional Biosafety Committee members are appropriately trained. The Principal Investigator is responsible for ensuring that laboratory staff are appropriately trained. The institution is responsible for ensuring that the Principal Investigator has sufficient training; however, this responsibility may be delegated to the Institutional Biosafety Committee.

Section IV-B-1-i. Determine the necessity for health surveillance of personnel involved in connection with individual recombinant DNA projects; and if appropriate, conduct a health surveillance program for such projects. The institution shall establish and maintain a health surveillance program for personnel engaged in large-scale research or production activities involving viable organisms containing recombinant DNA molecules which require BL3 containment at the laboratory scale. The institution shall establish and maintain a health surveillance program for personnel engaged in animal research involving viable recombinant DNA-containing microorganisms that require BL3 or greater containment in the laboratory. The *Laboratory Safety Monograph* discusses various components of such a program (e.g., records of agents handled, active investigation of relevant illnesses, and the maintenance of serial serum samples for monitoring serologic changes that may result from the employees' work experience). Certain medical conditions may place a laboratory worker at increased risk in any endeavor where infectious agents are handled. Examples cited in the *Laboratory Safety Monograph* include gastrointestinal disorders and treatment with steroids, immunosuppressive drugs, or antibiotics. Workers with such disorders or treatment should be evaluated to determine whether they should be engaged in research with potentially hazardous organisms during their treatment or illness. Copies of the *Laboratory Safety Monograph* are available from the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

Section IV-B-1-j. Report any significant problems, violations of the *NIH Guidelines*, or any significant research-related accidents and illnesses to NIH/OBA within thirty days, unless the institution determines that a report has already been filed by the Principal Investigator or Institutional Biosafety Committee. Reports shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

Section IV-B-2. Institutional Biosafety Committee (IBC)

The institution shall establish an Institutional Biosafety Committee whose responsibilities need not be restricted to recombinant DNA. The Institutional Biosafety Committee shall meet the following requirements:

Section IV-B-2-a. Membership and Procedures

Section IV-B-2-a-(1). The Institutional Biosafety Committee must be comprised of no fewer than five members so selected that they collectively have experience and expertise in recombinant DNA technology and the capability to assess the safety of recombinant DNA research and to identify any potential risk to public health or the environment. At least two members shall not be affiliated with the institution (apart from their membership on the Institutional Biosafety Committee) and who represent the interest of the surrounding community with respect to health and protection of the environment (e.g., officials of state or local public health or environmental protection agencies, members of other local governmental bodies, or persons active in medical, occupational health, or environmental concerns in the community). The Institutional Biosafety Committee shall include at least one individual with expertise in plant, plant pathogen, or plant pest containment principles when experiments utilizing [Appendix P](#), *Physical and Biological Containment for Recombinant DNA Research*

Involving Plants, require prior approval by the Institutional Biosafety Committee. The Institutional Biosafety Committee shall include at least one scientist with expertise in animal containment principles when experiments utilizing [Appendix Q, Physical and Biological Containment for Recombinant DNA Research Involving Animals](#), require Institutional Biosafety Committee prior approval. When the institution conducts recombinant DNA research at BL3, BL4, or Large Scale (greater than 10 liters), a Biological Safety Officer is mandatory and shall be a member of the Institutional Biosafety Committee (see [Section IV-B-3, Biological Safety Officer](#)). When the institution participates in or sponsors recombinant DNA research involving human research participants, the institution must ensure that: (i) the Institutional Biosafety Committee has adequate expertise and training (using *ad hoc* consultants as deemed necessary); (ii) all aspects of [Appendix M](#) have been appropriately addressed by the Principal Investigator; (iii) no research participant shall be enrolled (see definition of enrollment in [Section I-E-7](#)) in a human gene transfer experiment until the RAC review process has been completed (see [Appendix M-I-B, RAC Review Requirements](#)); and (iv) final IBC approval is granted only after the RAC review process has been completed (see [Appendix M-I-B, RAC Review Requirements](#)). Institutional Biosafety Committee approval must be obtained from the institution at which recombinant DNA material will be administered to human research participants (rather than the site involved in manufacturing gene transfer products).

Note: Individuals, corporations, and institutions not otherwise covered by the *NIH Guidelines*, are encouraged to adhere to the standards and procedures set forth in [Sections I](#) through [IV](#) (see [Section IV-D, Voluntary Compliance](#). The policy and procedures for establishing an Institutional Biosafety Committee under *Voluntary Compliance*, are specified in [Section IV-D-2, Institutional Biosafety Committee Approval](#)).

Section IV-B-2-a-(2). In order to ensure the competence necessary to review and approve recombinant DNA activities, it is recommended that the Institutional Biosafety Committee: (i) include persons with expertise in recombinant DNA technology, biological safety, and physical containment; (ii) include or have available as consultants persons knowledgeable in institutional commitments and policies, applicable law, standards of professional conduct and practice, community attitudes, and the environment, and (iii) include at least one member representing the laboratory technical staff.

Section IV-B-2-a-(3). The institution shall file an annual report with NIH/OBA which includes: (i) a roster of all Institutional Biosafety Committee members clearly indicating the Chair, contact person, Biological Safety Officer (if applicable), plant expert (if applicable), animal expert (if applicable), human gene therapy expertise or *ad hoc* consultant(if applicable); and (ii) biographical sketches of all Institutional Biosafety Committee members (including community members).

Section IV-B-2-a-(4). No member of an Institutional Biosafety Committee may be involved (except to provide information requested by the Institutional Biosafety Committee) in the review or approval of a project in which he/she has been or expects to be engaged or has a direct financial interest.

Section IV-B-2-a-(5). The institution, that is ultimately responsible for the effectiveness of the Institutional Biosafety Committee, may establish procedures that the Institutional Biosafety Committee shall follow in its initial and continuing review and approval of applications, proposals, and activities.

Section IV-B-2-a-(6). When possible and consistent with protection of privacy and proprietary interests, the institution is encouraged to open its Institutional Biosafety Committee meetings to the public.

Section IV-B-2-a-(7). Upon request, the institution shall make available to the public all Institutional Biosafety Committee meeting minutes and any documents submitted to or received from funding agencies which the latter are required to make available to the public. If public comments are made on Institutional Biosafety Committee actions, the institution shall forward both the public comments and the Institutional Biosafety Committee's response to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

Section IV-B-2-b. Functions

On behalf of the institution, the Institutional Biosafety Committee is responsible for:

Section IV-B-2-b-(1). Reviewing recombinant DNA research conducted at or sponsored by the institution for compliance with the *NIH Guidelines* as specified in [Section III, Experiments Covered by the NIH Guidelines](#), and approving those research projects that are found to conform with the *NIH Guidelines*. This review shall include: (i) independent assessment of the containment levels required by the *NIH Guidelines* for the proposed research; (ii) assessment of the facilities, procedures, practices, and training and expertise of personnel involved in recombinant DNA research; (iii) ensuring that all aspects of [Appendix M](#) have been appropriately addressed by the Principal Investigator; (iv) ensuring that no research participant is enrolled (see definition of enrollment in [Section I-E-7](#)) in a human gene transfer experiment until the RAC review process has been completed (see [Appendix M-I-B, RAC Review Requirements](#)), Institutional Biosafety Committee approval (from the clinical trial site) has been obtained, Institutional Review Board approval has been obtained, and all applicable regulatory authorizations have been obtained; (v) for human gene transfer protocols selected for public RAC review and discussion, consideration of the issues raised and recommendations made as a result of this review and consideration of the Principal Investigator's response to the RAC recommendations; (vi) ensuring that final IBC approval is granted only after the RAC review process has been completed (see [Appendix M-I-B, RAC Review Requirements](#)); and (vii) ensuring compliance with all surveillance, data reporting, and adverse event reporting requirements set forth in the *NIH Guidelines*.

Section IV-B-2-b-(2). Notifying the Principal Investigator of the results of the Institutional Biosafety Committee's review and approval.

Section IV-B-2-b-(3). Lowering containment levels for certain experiments as specified in [Section III-D-2-a, Experiments in which DNA from Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents is Cloned into Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems](#).

Section IV-B-2-b-(4). Setting containment levels as specified in [Sections III-D-4-b, Experiments Involving Whole Animals](#), and [III-D-5, Experiments Involving Whole Plants](#).

Section IV-B-2-b-(5). Periodically reviewing recombinant DNA research conducted at the institution to ensure compliance with the *NIH Guidelines*.

Section IV-B-2-b-(6). Adopting emergency plans covering accidental spills and personnel contamination resulting from recombinant DNA research.

Note: The *Laboratory Safety Monograph* describes basic elements for developing specific procedures dealing with major spills of potentially hazardous materials in the laboratory, including information and references about decontamination and emergency plans. The NIH and the Centers for Disease Control and Prevention are available to provide consultation and direct assistance, if necessary, as posted in the *Laboratory Safety Monograph*. The institution shall cooperate with the state and local public health departments by reporting any significant research-related illness or accident that may be hazardous to the public health.

Section IV-B-2-b-(7). Reporting any significant problems with or violations of the *NIH Guidelines* and any significant research-related accidents or illnesses to the appropriate institutional official and NIH/OBA within 30 days, unless the Institutional Biosafety Committee determines that a report has already been filed by the Principal Investigator. Reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

Section IV-B-2-b-(8). The Institutional Biosafety Committee may not authorize initiation of experiments which are not explicitly covered by the *NIH Guidelines* until NIH (with the advice of the RAC when required) establishes the containment requirement.

Section IV-B-2-b-(9). Performing such other functions as may be delegated to the Institutional Biosafety Committee under [Section IV-B-2, Institutional Biosafety Committee](#).

Section IV-B-3. Biological Safety Officer (BSO)

Section IV-B-3-a. The institution shall appoint a Biological Safety Officer if it engages in large-scale research or production activities involving viable organisms containing recombinant DNA molecules.

Section IV-B-3-b. The institution shall appoint a Biological Safety Officer if it engages in recombinant DNA research at BL3 or BL4. The Biological Safety Officer shall be a member of the Institutional Biosafety Committee.

Section IV-B-3-c. The Biological Safety Officer's duties include, but are not be limited to:

Section IV-B-3-c-(1). Periodic inspections to ensure that laboratory standards are rigorously followed;

Section IV-B-3-c-(2). Reporting to the Institutional Biosafety Committee and the institution any significant problems, violations of the *NIH Guidelines*, and any significant research-related accidents or illnesses of which the Biological Safety Officer becomes aware unless the Biological Safety Officer determines that a report has already been filed by the Principal Investigator;

Section IV-B-3-c-(3). Developing emergency plans for handling accidental spills and personnel contamination and investigating laboratory accidents involving recombinant DNA research;

Section IV-B-3-c-(4). Providing advice on laboratory security;

Section IV-B-3-c-(5). Providing technical advice to Principal Investigators and the Institutional Biosafety Committee on research safety procedures.

Note: See the *Laboratory Safety Monograph* for additional information on the duties of the Biological Safety Officer.

Section IV-B-4. Plant, Plant Pathogen, or Plant Pest Containment Expert

When the institution conducts recombinant DNA research that requires Institutional Biosafety Committee approval in accordance with [Appendix P](#), *Physical and Biological Containment for Recombinant DNA Research Involving Plants*, the institution shall appoint at least one individual with expertise in plant, plant pathogen, or plant pest containment principles (who is a member of the Institutional Biosafety Committee).

Section IV-B-5. Animal Containment Expert

When the institution conducts recombinant DNA research that requires Institutional Biosafety Committee approval in accordance with [Appendix Q](#), *Physical and Biological Containment for Recombinant DNA Research Involving Animals*, the institution shall appoint at least one individual with expertise in animal containment principles (who is a member of the Institutional Biosafety Committee).

Section IV-B-6. Human Gene Therapy Expertise

When the institution participates in or sponsors recombinant DNA research involving human subjects, the institution must ensure that: (i) the Institutional Biosafety Committee has adequate expertise and training (using *ad hoc* consultants as deemed necessary) and (ii) all aspects of [Appendix M](#), *Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA Molecules into One or More Human Subjects (Points to Consider)*, have been appropriately addressed by the Principal Investigator prior to submission to NIH/OBA.

Section IV-B-7. Principal Investigator (PI)

On behalf of the institution, the Principal Investigator is responsible for full compliance with the *NIH Guidelines* in the conduct of recombinant DNA research. A Principal Investigator engaged in human gene transfer research may delegate to another party, such as a corporate sponsor, the reporting functions set forth in [Appendix M](#), with written notification to the NIH OBA of the delegation and of the name(s), address, telephone, and fax numbers of the contact. The Principal Investigator is responsible for ensuring that the reporting requirements are fulfilled and will be held accountable for any reporting lapses.

Section IV-B-7-a. General Responsibilities

As part of this general responsibility, the Principal Investigator shall:

Section IV-B-7-a-(1). Initiate or modify no recombinant DNA research which requires Institutional Biosafety Committee approval prior to initiation (see Sections [III-A](#), [III-B](#), [III-C](#), [III-D](#), and [III-E](#), *Experiments Covered by the NIH Guidelines*) until that research or the proposed modification thereof has been approved by the Institutional Biosafety Committee and has met all other requirements of the *NIH Guidelines*;

Section IV-B-7-a-(2). Determine whether experiments are covered by [Section III-E](#), *Experiments that Require Institutional Biosafety Committee Notice Simultaneous with Initiation*, and ensure that the appropriate procedures are followed;

Section IV-B-7-a-(3). Report any significant problems, violations of the *NIH Guidelines*, or any significant research-related accidents and illnesses to the Biological Safety Officer (where applicable), Greenhouse/Animal Facility Director (where applicable), Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities (if applicable) within 30 days. Reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax);

Section IV-B-7-a-(4). Report any new information bearing on the *NIH Guidelines* to the Institutional Biosafety Committee and to NIH/OBA (reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax);

Section IV-B-7-a-(5). Be adequately trained in good microbiological techniques;

Section IV-B-7-a-(6). Adhere to Institutional Biosafety Committee approved emergency plans for handling accidental spills and personnel contamination; and

Section IV-B-7-a-(7). Comply with shipping requirements for recombinant DNA molecules (see [Appendix H](#), *Shipment*, for shipping requirements and the *Laboratory Safety Monograph* for technical recommendations).

Section IV-B-7-b. Information to Be Submitted by the Principal Investigator to NIH OBA

The Principal Investigator shall:

Section IV-B-7-b-(1). Submit information to NIH/OBA for certification of new host-vector systems;

Section IV-B-7-b-(2). Petition NIH/OBA, with notice to the Institutional Biosafety Committee, for proposed exemptions to the *NIH Guidelines*;

Section IV-B-7-b-(3). Petition NIH/OBA, with concurrence of the Institutional Biosafety Committee, for approval to conduct experiments specified in [Sections III-A-1](#), *Major Actions Under the NIH Guidelines*, and [III-B](#), *Experiments that Require NIH/OBA and Institutional Biosafety Committee Approval Before Initiation*;

Section IV-B-7-b-(4). Petition NIH/OBA for determination of containment for experiments requiring case-by-case review; and

Section IV-B-7-b-(5). Petition NIH/OBA for determination of containment for experiments not covered by the *NIH Guidelines*.

Section IV-B-7-b-(6). Ensure that all aspects of [Appendix M](#) have been appropriately addressed prior to submission of a human gene transfer experiment to NIH OBA, and provide a letter signed by the Principal Investigator(s) on institutional letterhead acknowledging that the documentation being submitted to NIH OBA complies with the requirements set forth in [Appendix M](#). No research participant shall be enrolled (see definition of enrollment in [Section I-E-7](#)) in a human gene transfer experiment until the RAC review process has been

completed (see [Appendix M-I-B](#), *RAC Review Requirements*); IBC approval (from the clinical trial site) has been obtained; Institutional Review Board (IRB) approval has been obtained; and all applicable regulatory authorization(s) have been obtained.

For a clinical trial site that is added after the RAC review process, no research participant shall be enrolled (see definition of enrollment in [Section I-E-7](#)) at the clinical trial site until the following documentation has been submitted to NIH OBA: (1) IBC approval (from the clinical trial site); (2) IRB approval; (3) IRB-approved informed consent document; (4) curriculum vitae of the principal investigator(s) (no more than two pages in biographical sketch format); and (5) NIH grant number(s) if applicable.

Section IV-B-7-c. Submissions by the Principal Investigator to the Institutional Biosafety Committee

The Principal Investigator shall:

Section IV-B-7-c-(1). Make an initial determination of the required levels of physical and biological containment in accordance with the *NIH Guidelines*;

Section IV-B-7-c-(2). Select appropriate microbiological practices and laboratory techniques to be used for the research;

Section IV-B-7-c-(3). Submit the initial research protocol and any subsequent changes (e.g., changes in the source of DNA or host-vector system), if covered under Sections [III-A](#), [III-B](#), [III-C](#), [III-D](#), or [III-E](#) (*Experiments Covered by the NIH Guidelines*), to the Institutional Biosafety Committee for review and approval or disapproval; and

Section IV-B-7-c-(4). Remain in communication with the Institutional Biosafety Committee throughout the conduct of the project.

Section IV-B-7-d. Responsibilities of the Principal Investigator Prior to Initiating Research

The Principal Investigator shall:

Section IV-B-7-d-(1). Make available to all laboratory staff the protocols that describe the potential biohazards and the precautions to be taken;

Section IV-B-7-d-(2). Instruct and train laboratory staff in: (i) the practices and techniques required to ensure safety, and (ii) the procedures for dealing with accidents; and

Section IV-B-7-d-(3). Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations or serum collection).

Section IV-B-7-e. Responsibilities of the Principal Investigator During the Conduct of the Research

The Principal Investigator shall:

Section IV-B-7-e-(1). Supervise the safety performance of the laboratory staff to ensure that the required safety practices and techniques are employed;

Section IV-B-7-e-(2). Investigate and report any significant problems pertaining to the operation and implementation of containment practices and procedures in writing to the Biological Safety Officer (where applicable), Greenhouse/Animal Facility Director (where applicable), Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities (if applicable) (reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax));

Section IV-B-7-e-(3). Correct work errors and conditions that may result in the release of recombinant DNA materials; and

Section IV-B-7-e-(4). Ensure the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., purity and genotypic and phenotypic characteristics).

Section IV-B-7-e-(5). Comply with reporting requirements for human gene transfer experiments conducted in compliance with the *NIH Guidelines* (see [Appendix M-I-C, Reporting Requirements](#)).

Section IV-C. Responsibilities of the National Institutes of Health (NIH)

Section IV-C-1. NIH Director

The NIH Director is responsible for: (i) establishing the *NIH Guidelines*, (ii) overseeing their implementation, and (iii) their final interpretation. The NIH Director has responsibilities under the *NIH Guidelines* that involve OBA and RAC. OBA's responsibilities under the *NIH Guidelines* are administrative. Advice from RAC is primarily scientific, technical, and ethical. In certain circumstances, there is specific opportunity for public comment with published response prior to final action.

Section IV-C-1-a. General Responsibilities

The NIH Director is responsible for:

Section IV-C-1-a-(1). Promulgating requirements as necessary to implement the *NIH Guidelines*;

Section IV-C-1-a-(2). Establishing and maintaining RAC to carry out the responsibilities set forth in Section IV-C-2, *Recombinant DNA Advisory Committee* (RAC membership is specified in its [charter](#) and in [Section IV-C-2](#));

Section IV-C-1-a-(3). Establishing and maintaining NIH/OBA to carry out the responsibilities defined in [Section IV-C-3, Office of Biotechnology Activities](#);

Section IV-C-1-a-(4). Conducting and supporting training programs in laboratory safety for Institutional Biosafety Committee members, Biological Safety Officers and other institutional experts (if applicable), Principal Investigators, and laboratory staff.

Section IV-C-1-a-(5). Establishing and convening Gene Therapy Policy Conferences as described in [Appendix L, Gene Therapy Policy Conferences](#).

Section IV-C-1-b. Specific Responsibilities

In carrying out the responsibilities set forth in this section, the NIH Director, or a designee shall weigh each proposed action through appropriate analysis and consultation to determine whether it complies with the *NIH Guidelines* and presents no significant risk to health or the environment.

Section IV-C-1-b-(1). Major Actions

To execute *Major Actions*, the NIH Director shall seek the advice of RAC and provide an opportunity for public and Federal agency comment. Specifically, the Notice of Meeting and *Proposed Actions* shall be published in the *Federal Register* at least 15 days before the RAC meeting. The NIH Director's decision/recommendation (at his/her discretion) may be published in the *Federal Register* for 15 days of comment before final action is taken. The NIH Director's final decision/recommendation, along with responses to public comments, shall be published in the *Federal Register*. The RAC and Institutional Biosafety Committee Chairs shall be notified of the following decisions:

Section IV-C-1-b-(1)-(a). Changing containment levels for types of experiments that are specified in the *NIH Guidelines* when a *Major Action* is involved;

Section IV-C-1-b-(1)-(b). Assigning containment levels for types of experiments that are not explicitly considered in the *NIH Guidelines* when a *Major Action* is involved;

Section IV-C-1-b-(1)-(c). Promulgating and amending a list of classes of recombinant DNA molecules to be exempt from the *NIH Guidelines* because they consist entirely of DNA segments from species that exchange DNA by known physiological processes or otherwise do not present a significant risk to health or the environment;

Section IV-C-1-b-(1)-(d). Permitting experiments specified by [Section III-A, Experiments that Require Institutional Biosafety Committee Approval, RAC Review, and NIH Director Approval Before Initiation](#);

Section IV-C-1-b-(1)-(e). Certifying new host-vector systems with the exception of minor modifications of already certified systems (the standards and procedures for certification are described in [Appendix I-II, Certification of Host-Vector Systems](#)). Minor modifications constitute (e.g., those of minimal or no consequence to the properties relevant to containment); and

Section IV-C-1-b-(1)-(f). Adopting other changes in the *NIH Guidelines*.

Section IV-C-1-b-(2). Minor Actions

NIH/OBA shall carry out certain functions as delegated to it by the NIH Director (see [Section IV-C-3, Office of Biotechnology Activities](#)). *Minor Actions* (as determined by NIH/OBA in consultation with the RAC Chair and one or more RAC members, as necessary) will be transmitted to RAC and Institutional Biosafety Committee Chairs:

Section IV-C-1-b-(2)-(a). Changing containment levels for experiments that are specified in [Section III, Experiments Covered by the NIH Guidelines](#) (except when a *Major Action* is involved);

Section IV-C-1-b-(2)-(b). Assigning containment levels for experiments not explicitly considered in the *NIH Guidelines*;

Section IV-C-1-b-(2)-(c). Revising the *Classification of Etiologic Agents* for the purpose of these *NIH Guidelines* (see [Section V-A, Footnotes and References of Sections I-IV](#)).

Section IV-C-1-b-(2)-(d). Interpreting the *NIH Guidelines* for experiments to which the *NIH Guidelines* do not specifically assign containment levels;

Section IV-C-1-b-(2)-(e). Setting containment under [Sections III-D-1-d, Experiments Using Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems](#), and [III-D-2-b, Experiments in which DNA from Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents is Cloned into Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems](#);

Section IV-C-1-b-(2)-(f). Approving minor modifications of already certified host-vector systems (the standards and procedures for such modifications are described in [Appendix I-II, Certification of Host-Vector Systems](#));

Section IV-C-1-b-(2)-(g). Decertifying already certified host-vector systems;

Section IV-C-1-b-(2)-(h). Adding new entries to the list of molecules toxic for vertebrates (see [Appendix F, Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates](#)); and

Section IV-C-1-b-(2)-(i). Determining appropriate containment conditions for experiments according to case precedents developed under [Section IV-C-1-b-\(2\)-\(c\)](#).

Section IV-C-2. Recombinant DNA Advisory Committee (RAC)

The RAC is responsible for carrying out the functions specified in the *NIH Guidelines*, as well as others specified in its charter or assigned by the Secretary of Health and Human Services or the NIH Director. The RAC membership and procedures, in addition to those set forth in the *NIH Guidelines*, are specified in the charter for the RAC which is filed as provided in the General Services Administration Federal Advisory Committee Management regulations, 41 CFR part 101-6, and is available on the OBA web site, <http://oba.od.nih.gov/oba/rac/RACCharter2009.pdf>. In the event of a conflict between the *NIH Guidelines* and the charter, the charter shall control.

The RAC will consist of not less than 15 voting members, including the Chair, appointed under the procedures of the NIH and the Department of Health and Human Services. The maximum number of voting members will be established in the charter of the RAC. At least a majority of the voting members must be knowledgeable in relevant scientific fields, e.g., molecular genetics, molecular biology, recombinant DNA research, including clinical gene transfer research. At least 4 members of the RAC must be knowledgeable in fields such as public health, laboratory safety, occupational health, protection of human subjects of research, the environment, ethics, law, public attitudes or related fields. Representatives of the Federal agencies listed in the charter shall serve as non-voting members. Nominations for RAC members may be submitted to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

All meetings of the RAC shall be announced in the *Federal Register*, including tentative agenda items, 15 days before the meeting. Final agendas, if modified, shall be available at least 72 hours before the meeting. No item defined as a *Major Action* under [Section IV-C-1-b-\(1\)](#) may be added to an agenda following *Federal Register* publication.

RAC shall be responsible for:

Section IV-C-2-a. Advising the NIH Director on the following actions: (1) Adopting changes in the *NIH Guidelines*. (2) Assigning containment levels, changing containment levels, and approving experiments considered as *Major Actions* under the *NIH Guidelines*, i.e., the deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally, if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture. (3) Promulgating and amending lists of classes of recombinant DNA molecules to be exempt from the *NIH Guidelines* because they consist entirely of DNA segments from species that exchange DNA by known physiological processes or otherwise do not present a significant risk to health or the environment. (4) Certifying new host-vector systems.

Section IV-C-2-b. Identifying novel human gene transfer experiments deserving of public discussion by the full RAC;

Section IV-C-2-c. Transmitting to the NIH Director specific comments/ recommendations about: (i) a specific human gene transfer experiment, or (ii) a category of human gene transfer experiments;

Section IV-C-2-d. Publicly reviewing human gene transfer clinical trial data and relevant information evaluated and summarized by NIH/OBA in accordance with the annual data reporting requirements;

Section IV-C-2-e. Identifying broad scientific, safety, social, and ethical issues relevant to gene therapy research as potential Gene Therapy Policy Conference topics;

Section IV-C-2-f. Identifying novel social and ethical issues relevant to specific human applications of gene transfer and recommending appropriate modifications to the *Points to Consider* that will provide guidance in the preparation of relevant Informed Consent documents; and

Section IV-C-2-g. Identifying novel scientific and safety issues relevant to specific human applications of gene transfer and recommending appropriate modifications to the *Points to Consider* that will provide guidance in the design and submission of human gene transfer clinical trials.

Section IV-C-3. Office of Biotechnology Activities (OBA)

OBA shall serve as a focal point for information on recombinant DNA activities and provide advice to all within and outside NIH including institutions, Biological Safety Officers, Principal Investigators, Federal agencies, state and local governments, and institutions in the private sector. OBA shall carry out such other functions as may be delegated to it by the NIH Director. OBA's responsibilities include (but are not limited to) the following:

Section IV-C-3-a. Serving as the focal point for public access to summary information pertaining to human gene transfer experiments;

Section IV-C-3-b. Serving as the focal point for data management of human gene transfer experiments;

Section IV-C-3-c. Administering the annual data reporting requirements (and subsequent review) for human gene transfer experiments (see [Appendix M-I-C, Reporting Requirements](#));

Section IV-C-3-d. Transmitting comments/recommendations arising from public RAC discussion of a novel human gene transfer experiment to the NIH Director. RAC recommendations shall be forwarded to the Principal Investigator, the sponsoring institution, and other DHHS components, as appropriate.

Section IV-C-3-e. Collaborating with Principal Investigators, Institutional Biosafety Committees, Institutional Review Boards, and other DHHS components (including FDA and the [Office for Human Research Protections](#)), to ensure human gene transfer experiment registration compliance in accordance with [Appendix M-I, Requirements for Protocol Submission, Review, and Reporting-Human Gene Transfer Experiments](#) of the *NIH Guidelines*.

Section IV-C-3-f. Administering Gene Therapy Policy Conferences as deemed appropriate by the NIH Director (see [Appendix L, Gene Therapy Policy Conferences](#)).

Section IV-C-3-g. Reviewing and approving experiments in conjunction with *ad hoc* experts involving the cloning of genes encoding for toxin molecules that are lethal for vertebrates at an LD₅₀ of less than or equal to 100 nanograms per kilogram body weight in organisms other than *Escherichia coli* K-12 (see [Section III-B-1, Experiments Involving the Cloning of Toxin Molecules with LD₅₀ of Less than 100 Nanograms Per Kilogram Body Weight](#), [Appendix F, Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates](#));

Section IV-C-3-h. Serving as the executive secretary of RAC;

Section IV-C-3-i. Publishing in the *Federal Register*:

Section IV-C-3-i-(1). Announcements of RAC meetings and tentative agendas at least 15 days in advance (Note: If the agenda for a RAC meeting is modified, OBA shall make the revised agenda available to anyone upon request in advance of the meeting);

Section IV-C-3-i-(2). Announcements of Gene Therapy Policy Conferences and tentative agendas at least 15 days in advance;

Section IV-C-3-i-(3). Proposed *Major Actions* (see [Section IV-C-1-b-\(1\), Major Actions](#)) at least 15 days prior to the RAC meeting; and

Section IV-C-3-j. Reviewing and approving the membership of an institution's Institutional Biosafety Committee, and where it finds the Institutional Biosafety Committee meets the requirements set forth in [Section IV-B-2, Institutional Biosafety Committee \(IBC\)](#), giving its approval to the Institutional Biosafety Committee membership.

Section IV-C-4. Other NIH Components

Other NIH components shall be responsible for certifying maximum containment (BL4) facilities, inspecting them periodically, and inspecting other recombinant DNA facilities as deemed necessary.

Section IV-D. Voluntary Compliance

Section IV-D-1. Basic Policy - Voluntary Compliance

Individuals, corporations, and institutions not otherwise covered by the *NIH Guidelines* are encouraged to follow the standards and procedures set forth in [Sections I](#) through IV. In order to simplify discussion, references hereafter to “institutions” are intended to encompass corporations and individuals who have no organizational affiliation. For purposes of complying with the *NIH Guidelines*, an individual intending to carry out research involving recombinant DNA is encouraged to affiliate with an institution that has an Institutional Biosafety Committee approved under the *NIH Guidelines*.

Since commercial organizations have special concerns, such as protection of proprietary data, some modifications and explanations of the procedures are provided in Sections IV-D-2 through IV-D-5-b, *Voluntary Compliance*, in order to address these concerns.

Section IV-D-2. Institutional Biosafety Committee Approval - Voluntary Compliance

It should be emphasized that employment of an Institutional Biosafety Committee member solely for purposes of membership on the Institutional Biosafety Committee does not itself make the member an institutionally affiliated member. Except for the unaffiliated members, a member of an Institutional Biosafety Committee for an institution not otherwise covered by the *NIH Guidelines* may participate in the review and approval of a project in which the member has a direct financial interest so long as the member has not been, and does not expect to be, engaged in the project. [Section IV-B-2-a\(4\)](#), *Institutional Biosafety Committee*, is modified to that extent for purposes of these institutions.

Section IV-D-3. Certification of Host-Vector Systems - Voluntary Compliance

A host-vector system may be proposed for certification by the NIH Director in accordance with the procedures set forth in [Appendix I-II](#), *Certification of Host-Vector Systems*. In order to ensure protection for proprietary data, any public notice regarding a host-vector system which is designated by the institution as proprietary under [Section IV-D](#), *Voluntary Compliance*, will be issued only after consultation with the institution as to the content of the notice.

Section IV-D-4. Requests for Exemptions and Approvals - Voluntary Compliance

Requests for exemptions or other approvals as required by the *NIH Guidelines* should be submitted based on the procedures set forth in [Sections I](#) through IV. In order to ensure protection for proprietary data, any public notice regarding a request for an exemption or other approval which is designated by the institution as proprietary under [Section IV-D-5-a](#), *Voluntary Compliance*, will be issued only after consultation with the institution as to the content of the notice.

Section IV-D-5. Protection of Proprietary Data - Voluntary Compliance

Section IV-D-5-a. General

In general, the Freedom of Information Act requires Federal agencies to make their records available to the public upon request. However, this requirement does not apply to, among other things, “trade secrets and commercial or financial information that is obtained from a person and that is privileged or confidential.” Under 18 U.S.C. 1905, it is a criminal offense for an officer or employee of the U.S. or any Federal department or agency to publish, divulge, disclose, or make known “in any manner or to any extent not authorized by law any information coming to him in the course of his employment or official duties or by reason of any examination or investigation made by, or return, report or record made to or filed with, such department or agency or officer or employee thereof, which information concerns or relates to the trade secrets, (or) processes...of any person, firm, partnership, corporation, or association.” This provision applies to all employees of the Federal Government, including special Government employees. Members of RAC are “special Government employees.”

In submitting to NIH for purposes of voluntary compliance with the *NIH Guidelines*, an institution may designate those items of information which the institution believes constitute trade secrets, privileged, confidential, commercial, or financial information. If NIH receives a request under the Freedom of Information Act for information so designated, NIH will promptly contact the institution to secure its views as to whether the information (or some portion) should be released. If NIH decides to release this information (or some portion) in response to a Freedom of Information request or otherwise, the institution will be advised and the actual release will be delayed in accordance with 45 Code of Federal Regulations, Section 5.65(d) and (e).

Section IV-D-5-b. Pre-submission Review

Any institution not otherwise covered by the *NIH Guidelines*, which is considering submission of data or information voluntarily to NIH, may request pre-submission review of the records involved to determine if NIH will make all or part of the records available upon request under the Freedom of Information Act.

A request for pre-submission review should be submitted to NIH/OBA along with the records involved to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). These records shall be clearly marked as being the property of the institution on loan to NIH solely for the purpose of making a determination under the Freedom on Information Act. NIH/OBA will seek a determination from the responsible official under DHHS regulations (45 CFR Part 5) as to whether the records involved, (or some portion) will be made available to members of the public under the Freedom of Information Act. Pending such a determination, the records will be kept separate from NIH/OBA files, will be considered records of the institution and not NIH/OBA, and will not be received as part of NIH/OBA files. No copies will be made of such records.

NIH/OBA will inform the institution of the NIH Freedom of Information Officer's determination and follow the institution's instructions as to whether some or all of the records involved are to be returned to the institution or to become a part of NIH/OBA files. If the institution instructs NIH/OBA to return the records, no copies or summaries of the records will be made or retained by DHHS, NIH, or OBA. The NIH Freedom of Information Officer's determination will represent that official's judgment at the time of the determination as to whether the records involved (or some portion) would be exempt from disclosure under the Freedom on Information Act if at the time of the determination the records were in NIH/OBA files and a request was received for such files under the Freedom of Information Act.

SECTION V. FOOTNOTES AND REFERENCES OF SECTIONS I THROUGH IV

Section V-A. The NIH Director, with advice of the RAC, may revise the classification for the purposes of the *NIH Guidelines* (see [Section IV-C-1-b-\(2\)-\(e\)](#), *Minor Actions*). The revised list of organisms in each risk group is reprinted in [Appendix B](#), *Classification of Human Etiologic Agents on the Basis of Hazard*.

Section V-B. [Section III](#), *Experiments Covered by the NIH Guidelines*, describes a number of places where judgments are to be made. In all these cases, the Principal Investigator shall make the judgment on these matters as part of his/her responsibility to "make the initial determination of the required levels of physical and biological containment in accordance with the *NIH Guidelines*" (see [Section IV-B-7-c-\(1\)](#)). For cases falling under [Sections III-A through III-E](#), *Experiments Covered by the NIH Guidelines*, this judgment is to be reviewed and approved by the Institutional Biosafety Committee as part of its responsibility to make an "independent assessment of the containment levels required by the *NIH Guidelines* for the proposed research" (see [Section IV-B-2-b-\(1\)](#), *Institutional Biosafety Committee*). The Institutional Biosafety Committee may refer specific cases to NIH/OBA as part of NIH/OBA's functions to "provide advice to all within and outside NIH" (see [Section IV-C-3](#),). NIH/OBA may request advice from the RAC as part of the RAC's responsibility for "interpreting the *NIH Guidelines* for experiments to which the *NIH Guidelines* do not specifically assign containment levels" (see [Section IV-C-1-b-\(2\)-\(f\)](#), *Minor Actions*).

Section V-C. U.S. Department of Health and Human Services, Public Health Service, [Centers for Disease Control and Prevention](#) and the [National Institutes of Health](#). *Biosafety in Microbiological and Biomedical Laboratories*, 5th Edition, 2007. Copies are available from: Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20401-0001, Phone (202) 512-1800 [<http://www.gpo.gov/>].

Section V-D. *Classification of Etiologic Agents on the Basis of Hazard*, 4th Edition, July 1974, U.S. Department of Health, Education, and Welfare, Public Health Service, Centers for Disease Control, Office of Biosafety, Atlanta, Georgia 30333.

Section V-E. Chin, James ed., *Control of Communicable Diseases Manual*, 17th Edition, 2000. ISBN: 087553-242-X, American Public Health Association, 800 I Street, N.W., Washington, D.C. Phone: (202) 777-2742.

Section V-F. *World Health Organization Laboratory Biosafety Manual*, 2nd edition. 1993. WHO Albany, NY. Copies are available from: WHO Publication Centre, USA, (Q Corp) 49 Sheridan Avenue, Albany, New York 12210; Phone: (518) 436-9686 (Order # 1152213).

Section V-G. A U.S. Department of Agriculture permit, required for import and interstate transport of plant and animal pathogens, may be obtained from the U.S. Department of Agriculture, ATTN: [Animal and Plant Health Inspection Service \(APHIS\)](#), Veterinary Services, National Center for Import-Export, Products Program, 4700 River Road, Unit 40, Riverdale, Maryland 20737. Phone: (301) 734-8499; Fax: (301) 734-8226.

Section V-H. American Type Culture Collection Catalogues of plant viruses, animal viruses, cells, bacteria, fungi, etc. are available from American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209. Phone: (703) 365-2700.

Section V-I. U.S. Department of Labor, [Occupational Safety and Health Administration](#), 29 CFR 1910.1030, *Bloodborne Pathogens*. See also, *Exposure to Bloodborne Pathogens*, OSHA 3127, 1996 (Revised).

Section V-J. As classified in the *Virus Taxonomy: The Classification and Nomenclature of Viruses. The Seventh Report of the International Committee on Taxonomy of Viruses*, Academic Press, 2000 (0123702003) San Diego, CA.

Section V-K. *i.e.*, the total of all genomes within a family shall not exceed two-thirds of the genome.

Section V-L. Organisms including alastrim, smallpox (variola) and whitepox may not be studied in the United States except at specified facilities. All activities, including storage of variola and whitepox, are restricted to the single national facility (World Health Organization Collaborating Center for Smallpox Research, [Centers for Disease Control and Prevention](#), Atlanta, Georgia).

Section V-M. In accordance with accepted scientific and regulatory practices of the discipline of plant pathology, an exotic plant pathogen (e.g., virus, bacteria, or fungus) is one that is unknown to occur within the U.S. (see [Section V-G](#), *Footnotes and References of Sections I-IV*). Determination of whether a pathogen has a potential for serious detrimental impact on managed (agricultural, forest, grassland) or natural ecosystems should be made by the Principal Investigator and the Institutional Biosafety Committee, in consultation with scientists knowledgeable of plant diseases, crops, and ecosystems in the geographic area of the research.

APPENDIX A. EXEMPTIONS UNDER SECTION III-F-5--SUBLISTS OF NATURAL EXCHANGERS

Certain specified recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent are exempt from these *NIH Guidelines* (see [Section III-F-5, Exempt Experiments](#)). Institutional Biosafety Committee registration is not required for these exempt experiments. A list of such exchangers will be prepared and periodically revised by the NIH Director with advice from the RAC after appropriate notice and opportunity for public comment (see [Section IV-C-1-b-\(1\)-\(c\), NIH Director--Specific Responsibilities](#)). For a list of natural exchangers that are exempt from the *NIH Guidelines*, see Appendices A-I through A-VI, *Exemptions Under Section III-F-5 Sublists of Natural Exchangers*. [Section III-F-5, Exempt Experiments](#), describes recombinant DNA molecules that are: (1) composed entirely of DNA segments from one or more of the organisms within a sublist, and (2) to be propagated in any of the organisms within a sublist (see *Classification of Bergey's Manual of Determinative Bacteriology*; 8th edition, R. E. Buchanan and N. E. Gibbons, editors, Williams and Wilkins Company; Baltimore, Maryland 1984). Although these experiments are exempt, it is recommended that they be performed at the appropriate biosafety level for the host or recombinant organism (see *Biosafety in Microbiological and Biomedical Laboratories*, 5th edition, 2007, U.S. DHHS, Public Health Service, [Centers for Disease Control and Prevention, Atlanta, Georgia](#), and NIH Office of Biosafety, Bethesda, Maryland).

Appendix A-I. Sublist A

Genus *Escherichia*
 Genus *Shigella*
 Genus *Salmonella* - including *Arizona*
 Genus *Enterobacter*
 Genus *Citrobacter* - including *Levinea*
 Genus *Klebsiella* - including *oxytoca*
 Genus *Erwinia*
Pseudomonas aeruginosa, *Pseudomonas putida*, *Pseudomonas fluorescens*, and *Pseudomonas mendocina*
Serratia marcescens
Yersinia enterocolitica

Appendix A-II. Sublist B

Bacillus subtilis
Bacillus licheniformis
Bacillus pumilus
Bacillus globigii
Bacillus niger
Bacillus nato
Bacillus amyloliquefaciens
Bacillus atterimus

Appendix A-III. Sublist C

Streptomyces aureofaciens
Streptomyces rimosus
Streptomyces coelicolor

Appendix A-IV. Sublist D

Streptomyces griseus
Streptomyces cyaneus
Streptomyces venezuelae

Appendix A-V. Sublist E

One way transfer of *Streptococcus mutans* or *Streptococcus lactis* DNA into *Streptococcus sanguis*

Appendix A-VI. Sublist F

Streptococcus sanguis
Streptococcus pneumoniae
Streptococcus faecalis
Streptococcus pyogenes
Streptococcus mutans

APPENDIX B. CLASSIFICATION OF HUMAN ETIOLOGIC AGENTS ON THE BASIS OF HAZARD

This appendix includes those biological agents known to infect humans as well as selected animal agents that may pose theoretical risks if inoculated into humans. Included are lists of representative genera and species known to be pathogenic; mutated, recombined, and non-pathogenic species and strains are not considered. Non-infectious life cycle stages of parasites are excluded.

This appendix reflects the current state of knowledge and should be considered a resource document. Included are the more commonly encountered agents and is not meant to be all-inclusive. Information on agent risk assessment may be found in the *Agent Summary Statements* of the CDC/NIH publication, *Biosafety in Microbiological and Biomedical Laboratories* (see [Sections V-C, V-D, V-E, and V-F](#), [Footnotes and References of Sections I through IV](#)). Further guidance on agents not listed in Appendix B may be obtained through: [Centers for Disease Control and Prevention](#), Biosafety Branch, Atlanta, Georgia 30333, Phone: (404) 639-3883, Fax: (404) 639-2294; National Institutes of Health, Division of Safety, Bethesda, Maryland 20892, Phone: (301) 496-1357; National Animal Disease Center, U.S. Department of Agriculture, Ames, Iowa 50010, Phone: (515) 862-8258.

A special committee of the American Society for Microbiology will conduct an annual review of this appendix and its recommendation for changes will be presented to the Recombinant DNA Advisory Committee as proposed amendments to the *NIH Guidelines*.

Appendix B - Table 1. Basis for the Classification of Biohazardous Agents by Risk Group (RG)

Risk Group 1 (RG1)	Agents that are not associated with disease in healthy adult humans
Risk Group 2 (RG2)	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are <i>often</i> available
Risk Group 3 (RG3)	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions <i>may be</i> available (high individual risk but low community risk)
Risk Group 4 (RG4)	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are <i>not usually</i> available (high individual risk and high community risk)

Appendix B-I. Risk Group 1 (RG1) Agents

RG1 agents are not associated with disease in healthy adult humans. Examples of RG1 agents include asporogenic *Bacillus subtilis* or *Bacillus licheniformis* (see [Appendix C-IV-A, Bacillus subtilis or Bacillus licheniformis Host-Vector Systems, Exceptions](#)); adeno- associated virus (AAV) types 1 through 4; and recombinant AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and are produced in the absence of a helper virus. A strain of *Escherichia coli* (see [Appendix C-II-A, Escherichia coli K-12 Host Vector Systems, Exceptions](#)) is an RG1 agent if it (1) does not possess a complete lipopolysaccharide (*i.e.*, lacks the O antigen); and (2) does not carry any active virulence factor (*e.g.*, toxins) or colonization factors and does not carry any genes encoding these factors.

Those agents not listed in Risk Groups (RGs) 2, 3 and 4 are not automatically or implicitly classified in RG1; a risk assessment must be conducted based on the known and potential properties of the agents and their relationship to agents that are listed.

Appendix B-II. Risk Group 2 (RG2) Agents

RG2 agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available.

Appendix B-II-A. Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia

- Acinetobacter baumannii* (formerly *Acinetobacter calcoaceticus*)
- Actinobacillus*
- Actinomyces pyogenes* (formerly *Corynebacterium pyogenes*)
- Aeromonas hydrophila*
- Amycolata autotrophica*
- Archanobacterium haemolyticum* (formerly *Corynebacterium haemolyticum*)
- Arizona hinshawii* - all serotypes
- Bacillus anthracis*
- Bartonella henselae*, *B. quintana*, *B. vinsonii*
- Bordetella* including *B. pertussis*
- Borrelia recurrentis*, *B. burgdorferi*
- Burkholderia* (formerly *Pseudomonas* species) except those listed in Appendix B-III-A (RG3)
- Campylobacter coli*, *C. fetus*, *C. jejuni*
- Chlamydia psittaci*, *C. trachomatis*, *C. pneumoniae*
- Clostridium botulinum*, *C. chauvoei*, *C. haemolyticum*, *C. histolyticum*, *C. novyi*, *C. septicum*, *C. tetani*
- Coxiella burnetii* – specifically the Phase II, Nine Mile strain, plaque purified, clone 4
- Corynebacterium diphtheriae*, *C. pseudotuberculosis*, *C. renale*
- Dermatophilus congolensis*
- Edwardsiella tarda*
- Erysipelothrix rhusiopathiae*
- Escherichia coli* - all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen, including *E. coli* O157:H7
- **Francisella tularensis* specifically **F. tularensis* subspecies *novocida* [aka *F. novocida*], strain Utah 112; **F. tularensis* subspecies *holartica* LVS; **F. tularensis* biovar *tularensis* strain ATCC 6223 (aka strain B38)
- Haemophilus ducreyi*, *H. influenzae*
- Helicobacter pylori*
- Klebsiella* - all species except *K. oxytoca* (RG1)
- Legionella* including *L. pneumophila*
- Leptospira interrogans* - all serotypes
- Listeria*
- Moraxella*
- Mycobacterium* (except those listed in [Appendix B-III-A](#) (RG3)) including *M. avium* complex, *M. asiaticum*, *M. bovis* BCG vaccine strain, *M. chelonae*, *M. fortuitum*, *M. kansasii*, *M. leprae*, *M. malmoense*, *M. marinum*, *M. paratuberculosis*, *M. scrofulaceum*, *M. simiae*, *M. szulgai*, *M. ulcerans*, *M. xenopi*
- Mycoplasma*, except *M. mycoides* and *M. agalactiae* which are restricted animal pathogens
- Neisseria gonorrhoeae*, *N. meningitidis*
- Nocardia asteroides*, *N. brasiliensis*, *N. otitidiscaviarum*, *N. transvalensis*
- Rhodococcus equi*
- Salmonella* including *S. arizonae*, *S. cholerasuis*, *S. enteritidis*, *S. gallinarum-pullorum*, *S. meleagridis*, *S. paratyphi*, A, B, C, *S. typhi*, *S. typhimurium*
- Shigella* including *S. boydii*, *S. dysenteriae*, type 1, *S. flexneri*, *S. sonnei*
- Sphaerophorus necrophorus*
- Staphylococcus aureus*
- Streptobacillus moniliformis*

* For research involving high concentrations, BL3 practices should be considered (See [Appendix G-II-C-2. Special Practices](#) (BL3)).

- Streptococcus* including *S. pneumoniae*, *S. pyogenes*
- Treponema pallidum*, *T. carateum*
- Vibrio cholerae*, *V. parahemolyticus*, *V. vulnificus*
- Yersinia enterocolitica*
- Yersinia pestis* specifically *pgm*⁽⁻⁾ strains (lacking the 102 kb pigmentation locus) and *lcr*⁽⁻⁾ strains (lacking the LCR plasmid).

Appendix B-II-B. Risk Group 2 (RG2) - Fungal Agents

- Blastomyces dermatitidis*
- Cladosporium bantianum*, *C. (Xylohypha) trichoides*
- Cryptococcus neoformans*
- Dactylaria galopava (Ochroconis gallopavum)*
- Epidermophyton*
- Exophiala (Wangiella) dermatitidis*
- Fonsecaea pedrosoi*
- Microsporium*
- Paracoccidioides braziliensis*
- Penicillium marneffeii*
- Sporothrix schenckii*
- Trichophyton*

Appendix B-II-C. Risk Group 2 (RG2) - Parasitic Agents

- Ancylostoma* human hookworms including *A. duodenale*, *A. ceylanicum*
- Ascaris* including *Ascaris lumbricoides suum*
- Babesia* including *B. divergens*, *B. microti*
- Brugia* filaria worms including *B. malayi*, *B. timori*
- Coccidia*
- Cryptosporidium* including *C. parvum*
- Cysticercus cellulosae* (hydatid cyst, larva of *T. solium*)
- Echinococcus* including *E. granulosus*, *E. multilocularis*, *E. vogeli*
- Entamoeba histolytica*
- Enterobius*
- Fasciola* including *F. gigantica*, *F. hepatica*
- Giardia* including *G. lamblia*
- Heterophyes*
- Hymenolepis* including *H. diminuta*, *H. nana*
- Isospora*
- Leishmania* including *L. braziliensis*, *L. donovani*, *L. ethiopia*, *L. major*, *L. mexicana*, *L. peruviana*, *L. tropica*
- Loa loa* filaria worms
- Microsporidium*
- Naegleria fowleri*
- Necator* human hookworms including *N. americanus*
- Onchocerca* filaria worms including, *O. volvulus*
- Plasmodium* including simian species, *P. cynomologi*, *P. falciparum*, *P. malariae*, *P. ovale*, *P. vivax*
- Sarcocystis* including *S. sui hominis*
- Schistosoma* including *S. haematobium*, *S. intercalatum*, *S. japonicum*, *S. mansoni*, *S. mekongi*
- Strongyloides* including *S. stercoralis*
- Taenia solium*
- Toxocara* including *T. canis*
- Toxoplasma* including *T. gondii*
- Trichinella spiralis*
- Trypanosoma* including *T. brucei brucei*, *T. brucei gambiense*, *T. brucei rhodesiense*, *T. cruzi*
- Wuchereria bancrofti* filaria worms

Appendix B-II-D. Risk Group 2 (RG2) - Viruses

Adenoviruses, human - all types

Alphaviruses (Togaviruses) - Group A Arboviruses

- Chikungunya vaccine strain 181/25
- Eastern equine encephalomyelitis virus
- Venezuelan equine encephalomyelitis vaccine strains TC-83 and V3526
- Western equine encephalomyelitis virus

Arenaviruses

- Junin virus candid #1 vaccine strain
- Lymphocytic choriomeningitis virus (non-neurotropic strains)
- Tacaribe virus complex
- Other viruses as listed in the reference source (see [Section V-C, Footnotes and References of Sections I through IV](#))

Bunyaviruses

- Bunyamwera virus
- Rift Valley fever virus vaccine strain MP-12
- Other viruses as listed in the reference source (see [Section V-C, Footnotes and References of Sections I through IV](#))

Caliciviruses

Coronaviruses

Flaviviruses - Group B Arboviruses

- Dengue virus serotypes 1, 2, 3, and 4
- Japanese encephalitis virus strain SA 14-14-2
- Yellow fever virus vaccine strain 17D
- Other viruses as listed in the reference source (see [Section V-C, Footnotes and References of Sections I through IV](#))

Hepatitis A, B, C, D, and E viruses

Herpesviruses - except Herpesvirus simiae (Monkey B virus) (see [Appendix B-IV-D, Risk Group 4 \(RG4\) - Viral Agents](#))

- Cytomegalovirus
- Epstein Barr virus
- Herpes simplex* types 1 and 2
- Herpes zoster*
- Human herpesvirus types 6 and 7

Orthomyxoviruses

- Influenza viruses types A, B, and C (except those listed in [Appendix B-III-D, Risk Group 3 \(RG3\) - Viruses and Prions](#))
- Tick-borne orthomyxoviruses

Papilloma viruses

- All human papilloma viruses

Paramyxoviruses

- Newcastle disease virus
- Measles virus
- Mumps virus
- Parainfluenza viruses types 1, 2, 3, and 4
- Respiratory syncytial virus

Parvoviruses

- Human parvovirus (B19)

Picornaviruses

- Coxsackie viruses types A and B
- Echoviruses - all types
- Polioviruses - all types, wild and attenuated
- Rhinoviruses - all types

Poxviruses - all types except Monkeypox virus (see [Appendix B-III-D, Risk Group 3 \(RG3\) - Viruses and Prions](#)) and restricted poxviruses including Alastrim, Smallpox, and Whitepox (see [Section V-L, Footnotes and References of Sections I through IV](#))

Reoviruses - all types including Coltivirus, human Rotavirus, and Orbivirus (Colorado tick fever virus)

Rhabdoviruses

- Rabies virus - all strains
- Vesicular stomatitis virus non exotic strains: VSV-Indiana 1 serotype strains (e.g. Glasgow, Mudd-Summers, Orsay, San Juan) and VSV-New Jersey serotype strains (e.g. Ogden, Hazelhurst)

Rubivirus (Togaviruses)

- Rubella virus

Appendix B-III. Risk Group 3 (RG3) Agents

RG3 agents are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available.

Appendix B-III-A. Risk Group 3 (RG3) - Bacterial Agents Including Rickettsia

- Bartonella*
- Brucella* including *B. abortus*, *B. canis*, *B. suis*
- Burkholderia (Pseudomonas) mallei*, *B. pseudomallei*
- Coxiella burnetii* (except the Phase II, Nine Mile strain listed in [Appendix B-II-A, Risk Group 2 \(RG2\) - Bacterial Agents Including Chlamydia](#))
- Francisella tularensis* (except those strains listed in [Appendix B-II-A, Risk Group 2 \(RG2\) - Bacterial Agents Including Chlamydia](#))
- Mycobacterium bovis* (except BCG strain, see [Appendix B-II-A, Risk Group 2 \(RG2\) - Bacterial Agents Including Chlamydia](#)), *M. tuberculosis*
- Pasteurella multocida* type B -"buffalo" and other virulent strains
- Rickettsia akari*, *R. australis*, *R. canada*, *R. conorii*, *R. prowazekii*, *R. rickettsii*, *R. siberica*, *R. tsutsugamushi*, *R. typhi* (*R. mooseri*)
- Yersinia pestis* (except those strains listed in [Appendix B-II-A, Risk Group 2 \(RG2\) - Bacterial Agents Including Chlamydia](#))

Appendix B-III-B. Risk Group 3 (RG3) - Fungal Agents

- Coccidioides immitis* (sporulating cultures; contaminated soil)
- Histoplasma capsulatum*, *H. capsulatum* var. *duboisii*

Appendix B-III-C. Risk Group 3 (RG3) - Parasitic Agents

None

Appendix B-III-D. Risk Group 3 (RG3) - Viruses and Prions

Alphaviruses (Togaviruses) - Group A Arboviruses

- Chikungunya virus (except the vaccine strain 181/25 listed in [Appendix B-II-D Risk Group2 \(RG2\) – Viruses](#))
- Semliki Forest virus
- St. Louis encephalitis virus
- Venezuelan equine encephalomyelitis virus (except the vaccine strains TC-83 and V3526, see [Appendix B-II-D \(RG2\) – Viruses](#))

--Other viruses as listed in the reference source (see [Section V-C, Footnotes and References of Sections I through IV](#))

Arenaviruses

- Flexal
- Lymphocytic choriomeningitis virus (LCM) (neurotropic strains)

Bunyaviruses

- Hantaviruses including Hantaan virus
- Rift Valley fever virus

Coronaviruses

- SARS-associated coronavirus (SARS-CoV)

Flaviviruses - Group B Arboviruses

- Japanese encephalitis virus (except those strains listed in [Appendix B-II-D Risk Group2 \(RG2\) - Viruses](#))
- West Nile virus (WNV)
- Yellow fever virus
- Other viruses as listed in the reference source (see [Section V-C, Footnotes and References of Sections I through IV](#))

Orthomyxoviruses

- Influenza viruses 1918-1919 H1N1 (1918 H1N1), human H2N2 (1957-1968), and highly pathogenic avian influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1).

Poxviruses

- Monkeypox virus

Prions

- Transmissible spongiform encephalopathies (TME) agents (Creutzfeldt-Jacob disease and kuru agents)(see [Section V-C, Footnotes and References of Sections I through IV](#), for containment instruction)

Retroviruses

- Human immunodeficiency virus (HIV) types 1 and 2
- Human T cell lymphotropic virus (HTLV) types 1 and 2
- Simian immunodeficiency virus (SIV)

Rhabdoviruses

- Vesicular stomatitis virus (except those strains listed in [Appendix B-II-D Risk Group2 \(RG2\) - Viruses](#))

Appendix B-IV. Risk Group 4 (RG4) Agents

RG4 agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available.

Appendix B-IV-A. Risk Group 4 (RG4) - Bacterial Agents

None

Appendix B-IV-B. Risk Group 4 (RG4) - Fungal Agents

None

Appendix B-IV-C. Risk Group 4 (RG4) - Parasitic Agents

None

Appendix B-IV-D. Risk Group 4 (RG4) - Viral Agents

Arenaviruses

--Guanarito virus
 --Lassa virus

--Junin virus (except the candid #1 vaccine strain listed in [Appendix B-II-D Risk Group2 \(RG2\) – Viruses](#))
 --Machupo virus
 --Sabia

Bunyaviruses (Nairovirus)
 --Crimean-Congo hemorrhagic fever virus

Filoviruses
 --Ebola virus
 --Marburg virus

Flaviruses - Group B Arboviruses
 --Tick-borne encephalitis virus complex including Absetterov, Central European encephalitis, Hanzalova, Hypr, Kumlinge, Kyasanur Forest disease, Omsk hemorrhagic fever, and Russian spring-summer encephalitis viruses

Herpesviruses (alpha)
 --Herpesvirus simiae (Herpes B or Monkey B virus)

Paramyxoviruses
 --Equine morbillivirus

Hemorrhagic fever agents and viruses as yet undefined

Appendix B-V. Animal Viral Etiologic Agents in Common Use

The following list of animal etiologic agents is appended to the list of human etiologic agents. None of these agents is associated with disease in healthy adult humans; they are commonly used in laboratory experimental work.

A containment level appropriate for RG1 human agents is recommended for their use. For agents that are infectious to human cells, e.g., amphotropic and xenotropic strains of murine leukemia virus, a containment level appropriate for RG2 human agents is recommended.

Baculoviruses

Herpesviruses
 --Herpesvirus ateles
 --Herpesvirus saimiri
 --Marek's disease virus
 --Murine cytomegalovirus

Papilloma viruses
 --Bovine papilloma virus
 --Shope papilloma virus

Polyoma viruses
 --Polyoma virus
 --Simian virus 40 (SV40)

Retroviruses
 --Avian leukosis virus
 --Avian sarcoma virus
 --Bovine leukemia virus
 --Feline leukemia virus
 --Feline sarcoma virus
 --Gibbon leukemia virus
 --Mason-Pfizer monkey virus

- Mouse mammary tumor virus
- Murine leukemia virus

- Murine sarcoma virus
- Rat leukemia virus

Appendix B-V-1. Murine Retroviral Vectors

Murine retroviral vectors to be used for human transfer experiments (less than 10 liters) that contain less than 50% of their respective parental viral genome and that have been demonstrated to be free of detectable replication competent retrovirus can be maintained, handled, and administered, under BL1 containment.

APPENDIX C. EXEMPTIONS UNDER SECTION III-F-6

Section III-F-6 states that exempt from these *NIH Guidelines* are "those that do not present a significant risk to health or the environment (see Section IV-C-1-b-(1)-(c), *NIH Director--Specific Responsibilities*), as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment. See Appendix C, *Exemptions under Sections III-F-6*, for other classes of experiments which are exempt from the *NIH Guidelines*." The following classes of experiments are exempt under Section III-F-6:

Appendix C-I. Recombinant DNA in Tissue Culture

Recombinant DNA molecules containing less than one-half of any eukaryotic viral genome (all viruses from a single family being considered identical -- see Appendix C-VIII-E, *Footnotes and References of Appendix C*), that are propagated and maintained in cells in tissue culture are exempt from these *NIH Guidelines* with the exceptions listed in Appendix C-I-A.

Appendix C-I-A. Exceptions

The following categories are not exempt from the *NIH Guidelines*: (i) experiments described in Section III-B which require NIH/OBA and Institutional Biosafety Committee approval before initiation, (ii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*, and Sections V-G and V-L, *Footnotes and References of Sections I through IV*) or cells known to be infected with these agents, (iii) experiments involving the deliberate introduction of genes coding for the biosynthesis of molecules that are toxic for vertebrates (see Appendix F, *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*), and (iv) whole plants regenerated from plant cells and tissue cultures are covered by the exemption provided they remain axenic cultures even though they differentiate into embryonic tissue and regenerate into plantlets.

Appendix C-II. *Escherichia coli* K-12 Host-Vector Systems

Experiments which use *Escherichia coli* K-12 host-vector systems, with the exception of those experiments listed in Appendix C-II-A, are exempt from the *NIH Guidelines* provided that: (i) the *Escherichia coli* host does not contain conjugation proficient plasmids or generalized transducing phages; or (ii) lambda or lambdoid or Ff bacteriophages or non-conjugative plasmids (see Appendix C-VIII-B, *Footnotes and References of Appendix C*) shall be used as vectors. However, experiments involving the insertion into *Escherichia coli* K-12 of DNA from prokaryotes that exchange genetic information (see Appendix C-VIII-C, *Footnotes and References of Appendix C*) with *Escherichia coli* may be performed with any *Escherichia coli* K-12 vector (e.g., conjugative plasmid). When a non-conjugative vector is used, the *Escherichia coli* K-12 host may contain conjugation-proficient plasmids either autonomous or integrated, or generalized transducing phages. For these exempt laboratory experiments, Biosafety Level (BL) 1 physical containment conditions are recommended. For large-scale fermentation experiments, the appropriate physical containment conditions need be no greater than those for the host organism unmodified by recombinant DNA techniques; the Institutional Biosafety Committee can specify higher containment if deemed necessary.

Appendix C-II-A. Exceptions

The following categories are not exempt from the *NIH Guidelines*: (i) experiments described in Section III-B

which require NIH/OBA and Institutional Biosafety Committee approval before initiation, (ii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see [Appendix B, Classification of Human](#)

Etiologic Agents on the Basis of Hazard, and [Sections V-G and V-L, Footnotes and References of Sections I through IV](#)) or cells known to be infected with these agents may be conducted under containment conditions specified in [Section III-D-2](#) with prior Institutional Biosafety Committee review and approval, (iii) large-scale experiments (e.g., more than 10 liters of culture), and (iv) experiments involving the cloning of toxin molecule genes coding for the biosynthesis of molecules toxic for vertebrates (see [Appendix F, Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates](#)).

Appendix C-III. *Saccharomyces* Host-Vector Systems

Experiments involving *Saccharomyces cerevisiae* and *Saccharomyces uvarum* host-vector systems, with the exception of experiments listed in Appendix C-III-A, are exempt from the *NIH Guidelines*. For these exempt experiments, BL1 physical containment is recommended. For large-scale fermentation experiments, the appropriate physical containment conditions need be no greater than those for the host organism unmodified by recombinant DNA techniques; the Institutional Biosafety Committee can specify higher containment if deemed necessary.

Appendix C-III-A. Exceptions

The following categories are not exempt from the *NIH Guidelines*: (i) experiments described in [Section III-B](#) which require NIH/OBA and Institutional Biosafety Committee approval before initiation, (ii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see [Appendix B, Classification of Human Etiologic Agents on the Basis of Hazard](#), and [Sections V-G and V-L, Footnotes and References of Sections I through IV](#)) or cells known to be infected with these agents may be conducted under containment conditions specified in [Section III-D-2](#) with prior Institutional Biosafety Committee review and approval, (iii) large-scale experiments (e.g., more than 10 liters of culture), and (iv) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see [Appendix F, Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates](#)).

Appendix C-IV. *Kluyveromyces* Host-Vector Systems

Experiments involving *Kluyveromyces lactis* host-vector systems, with the exception of experiments listed in Appendix C-IV-A, are exempt from the *NIH Guidelines* provided laboratory-adapted strains are used (i.e. strains that have been adapted to growth under optimal or defined laboratory conditions). For these exempt experiments, BL1 physical containment is recommended. For large-scale fermentation experiments, the appropriate physical containment conditions need be no greater than those for the host organism unmodified by recombinant DNA techniques; the Institutional Biosafety Committee may specify higher containment if deemed necessary.

Appendix C-IV-A. Exceptions

The following categories are not exempt from the *NIH Guidelines*: (i) experiments described in [Section III-B](#), which require NIH/OBA and Institutional Biosafety Committee approval before initiation; (ii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see [Appendix B, Classification of Human Etiologic Agents on the Basis of Hazard](#), and [Sections V-G and V-L, Footnotes and References of Sections I through IV](#)) or cells known to be infected with these agents may be conducted under containment conditions specified in [Section III-D-2](#) with prior Institutional Biosafety Committee review and approval; (iii) large-scale experiments (e.g., more than 10 liters of culture), and (v) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see [Appendix F, Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates](#)).

Appendix C-V. *Bacillus subtilis* or *Bacillus licheniformis* Host-Vector Systems

Any asporogenic *Bacillus subtilis* or asporogenic *Bacillus licheniformis* strain which does not revert to a spore-former with a frequency greater than 10^{-7} may be used for cloning DNA with the exception of those experiments listed in Appendix C-V-A, *Exceptions*. For these exempt laboratory experiments, BL1 physical containment conditions are recommended. For large-scale fermentation experiments, the appropriate physical containment

conditions need be no greater than those for the host organism unmodified by recombinant DNA techniques; the Institutional Biosafety Committee can specify higher containment if it deems necessary.

Appendix C-V-A. Exceptions

The following categories are not exempt from the *NIH Guidelines*: (i) experiments described in [Section III-B](#) which require NIH/OBA and Institutional Biosafety Committee approval before initiation, (ii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see [Appendix B, Classification of Human Etiologic Agents on the Basis of Hazard](#), and [Sections V-G and V-L, Footnotes and References of Sections I through IV](#)) or cells known to be infected with these agents may be conducted under containment conditions specified in [Section III-D-2](#) with prior Institutional Biosafety Committee review and approval, (iii) large-scale experiments (e.g., more than 10 liters of culture), and (iv) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see [Appendix F, Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates](#)).

Appendix C-VI. Extrachromosomal Elements of Gram Positive Organisms

Recombinant DNA molecules derived entirely from extrachromosomal elements of the organisms listed below (including shuttle vectors constructed from vectors described in [Appendix C](#)), propagated and maintained in organisms listed below are exempt from these *NIH Guidelines*.

Bacillus amyloliquefaciens
Bacillus amylosacchariticus
Bacillus anthracis
Bacillus atterimus
Bacillus brevis
Bacillus cereus
Bacillus globigii
Bacillus licheniformis
Bacillus megaterium
Bacillus natto
Bacillus niger
Bacillus pumilus
Bacillus sphaericus
Bacillus stearothermophilis
Bacillus subtilis
Bacillus thuringiensis
Clostridium acetobutylicum
Lactobacillus casei
Listeria grayi
Listeria monocytogenes
Listeria murrayi
Pediococcus acidilactici
Pediococcus damnosus
Pediococcus pentosaceus
Staphylococcus aureus
Staphylococcus carnosus
Staphylococcus epidermidis
Streptococcus agalactiae
Streptococcus anginosus
Streptococcus avium
Streptococcus cremoris
Streptococcus dorans
Streptococcus equisimilis
Streptococcus faecalis
Streptococcus ferus
Streptococcus lactis
Streptococcus ferns
Streptococcus mitior
Streptococcus mutans

Streptococcus pneumoniae
Streptococcus pyogenes

Streptococcus salivarius
Streptococcus sanguis
Streptococcus sobrinus
Streptococcus thermophilus

Appendix C-VI-A. Exceptions

The following categories are not exempt from the *NIH Guidelines*: (i) experiments described in [Section III-B](#) which require NIH/OBA and Institutional Biosafety Committee approval before initiation, (ii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see [Appendix B, Classification of Human Etiologic Agents on the Basis of Hazard](#), and [Sections V-G and V-L, Footnotes and References of Sections I through IV](#)) or cells known to be infected with these agents may be conducted under containment conditions specified in [Section III-D-2](#) with prior Institutional Biosafety Committee review and approval, (iii) large-scale experiments (e.g., more than 10 liters of culture), and (iv) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see [Appendix F, Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates](#)).

Appendix C-VII. The Purchase or Transfer of Transgenic Rodents

The purchase or transfer of transgenic rodents for experiments that require BL1 containment (See [Appendix G-III-M, Footnotes and References of Appendix G](#)) are exempt from the *NIH Guidelines*.

Appendix C-VIII. Generation of BL1 Transgenic Rodents via Breeding

The breeding of two different transgenic rodents or the breeding of a transgenic rodent and a non-transgenic rodent with the intent of creating a new strain of transgenic rodent that can be housed at BL1 containment will be exempt from the *NIH Guidelines* if:

- (1) Both parental rodents can be housed under BL1 containment; and
- (2) neither parental transgenic rodent contains the following genetic modifications: (i) incorporation of more than one-half of the genome of an exogenous eukaryotic virus from a single family of viruses; or (ii) incorporation of a transgene that is under the control of a gammaretroviral long terminal repeat (LTR); and
- (3) the transgenic rodent that results from this breeding is not expected to contain more than one-half of an exogenous viral genome from a single family of viruses.

Appendix C-IX. Footnotes and References of Appendix C

Appendix C-IX-A. The NIH Director, with advice of the RAC, may revise the classification for the purposes of these *NIH Guidelines* (see [Section IV-C-1-b-\(2\)-\(b\), Minor Actions](#)). The revised list of organisms in each Risk Group is located in [Appendix B](#).

Appendix C-IX-B. A subset of non-conjugative plasmid vectors are poorly mobilizable (e.g., pBR322, pBR313). Where practical, these vectors should be employed.

Appendix C-IX-C. Defined as observable under optimal laboratory conditions by transformation, transduction, phage infection, and/or conjugation with transfer of phage, plasmid, and/or chromosomal genetic information. Note that this definition of exchange may be less stringent than that applied to exempt organisms under [Section III-F-5, Exempt Experiments](#).

Appendix C-IX-D. As classified in the *Third Report of the International Committee on Taxonomy of Viruses: Classification and Nomenclature of Viruses*, R. E. F. Matthews (ed.), Intervirology 12 (129-296), 1979.

Appendix C-IX-E. i.e., the total of all genomes within a Family shall not exceed one-half of the genome.

APPENDIX D. MAJOR ACTIONS TAKEN UNDER THE NIH GUIDELINES

As noted in the subsections of [Section IV-C-1-b-\(1\)](#), the Director, NIH, may take certain actions with regard to the *NIH Guidelines* after the issues have been considered by the RAC. Some of the actions taken to date include the following:

Appendix D-1. Permission is granted to clone foot and mouth disease virus in the EK1 host-vector system consisting of *E. coli* K-12 and the vector pBR322, all work to be done at the Plum Island Animal Disease Center.

Appendix D-2. Certain specified clones derived from segments of the foot and mouth disease virus may be transferred from Plum Island Animal Disease Center to the facilities of Genentech, Inc., of South San Francisco, California. Further development of the clones at Genentech, Inc., has been approved under BL1 + EK1 conditions.

Appendix D-3. The Rd strain of *Hemophilus influenzae* can be used as a host for the propagation of the cloned Tn 10 tet R gene derived from *E. coli* K-12 employing the non-conjugative *Hemophilus* plasmid, pRSF0885, under BL1 conditions.

Appendix D-4. Permission is granted to clone certain subgenomic segments of foot and mouth disease virus in HV1 *Bacillus subtilis* and *Saccharomyces cerevisiae* host-vector systems under BL1 conditions at Genentech, Inc., South San Francisco, California.

Appendix D-5. Permission is granted to Dr. Ronald Davis of Stanford University to field test corn plants modified by recombinant DNA techniques under specified containment conditions.

Appendix D-6. Permission is granted to clone in *E. coli* K-12 under BL1 physical containment conditions subgenomic segments of rift valley fever virus subject to conditions which have been set forth by the RAC.

Appendix D-7. Attenuated laboratory strains of *Salmonella typhimurium* may be used under BL1 physical containment conditions to screen for the *Saccharomyces cerevisiae* pseudouridine synthetase gene. The plasmid YEp13 will be employed as the vector.

Appendix D-8. Permission is granted to transfer certain clones of subgenomic segments of foot and mouth disease virus from Plum Island Animal Disease Center to the laboratories of Molecular Genetics, Inc., Minnetonka, Minnesota, and to work with these clones under BL1 containment conditions. Approval is contingent upon review of data on infectivity testing of the clones by a working group of the RAC.

Appendix D-9. Permission is granted to Dr. John Sanford of Cornell University to field test tomato and tobacco plants transformed with bacterial (*E. coli* K-12) and yeast DNA using pollen as a vector.

Appendix D-10. Permission is granted to Drs. Steven Lindow and Nickolas Panopoulos of the University of California, Berkeley, to release under specified conditions *Pseudomonas syringae*, pathovars (pv.) *syringae*, and *Erwinia herbicola* carrying *in vitro* generated deletions of all or part of the genes involved in ice nucleation.

Appendix D-11. Agracetus of Middleton, Wisconsin, may field test under specified conditions disease resistant tobacco plants prepared by recombinant DNA techniques.

Appendix D-12. Eli Lilly and Company of Indianapolis, Indiana, may conduct large-scale experiments and production involving *Cephalosporium acremonium* strain LU4-79-6 under less than Biosafety Level 1 - Large Scale (BL1-LS) conditions.

Appendix D-13. Drs. W. French Anderson, R. Michael Blaese, and Steven Rosenberg of the NIH, Bethesda, Maryland, can conduct experiments in which a bacterial gene coding for neomycin phosphotransferase will be inserted into a portion of the tumor infiltrating lymphocytes (TIL) of cancer patients using a retroviral vector, N2. The marked TIL then will be combined with unmarked TIL, and reinfused into the patients. This experiment is an addition to an ongoing adoptive immunotherapy protocol in which TIL are isolated from a patient's tumor, grown in culture in the presence of interleukin-2, and reinfused into the patient. The marker gene will be used to detect TIL at various time intervals following reinfusion.

Approval is based on the following four stipulations: (i) there will be no limitation of the number of patients in the continuing trial; (ii) the patients selected will have a life expectancy of about 90 days; (iii) the patients give fully informed consent to participate in the trial; and (iv) the investigators will provide additional data before inserting a gene for therapeutic purposes. (Protocol #8810-001)

Appendix D-14. U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) may conduct certain experiments involving products of a yellow fever virus originating from the 17-D yellow fever clone at the Biosafety Level 3 containment level using HEPA filters and vaccination of laboratory personnel.

In addition, USAMRIID may conduct certain experiments involving vaccine studies of Venezuelan equine encephalitis virus at the Biosafety Level 3 containment level using HEPA filters and vaccination of laboratory personnel.

Appendix D-15. Drs. R. Michael Blaese and W. French Anderson of the NIH, Bethesda, Maryland, can conduct experiments in which a gene coding for adenosine deaminase (ADA) will be inserted into T lymphocytes of patients with severe combined immunodeficiency disease, using a retroviral vector, LASN. Following insertion of the gene, these T lymphocytes will be reinfused into the patients. The patients will then be followed for evidence of clinical improvement in the disease state, and measurement of multiple parameters of immune function by laboratory testing.

Approval is based on the following two stipulations: (i) that intraperitoneal administration of transduced T lymphocytes not be used before clearance by the Chair of the Recombinant DNA Advisory Committee; and (ii) that the number of research patients be limited to 10 at this time.

In addition to the conditions outlined in the initial approval, patients may be given a supplement of a CD-34+-enriched peripheral blood lymphocytes (PBL) which have been placed in culture conditions that favor progenitor cell growth. This enriched population of cells will be transduced with the retroviral vector, G1NaSvAd. G1NaSvAd is similar to LASN, yet distinguishable by PCR. LASN has been used to transduce peripheral blood T lymphocytes with the ADA gene. Lymphocytes and myeloid cells will be isolated from patients over time and assayed for the presence of the LASN or G1NaSvAd vectors. The primary objectives of this protocol are to transduce CD 34+ peripheral blood cells with the adenosine deaminase gene, administer these cells to patients, and determine if such cells can differentiate into lymphoid and myeloid cells *in vivo*. There is a potential for benefit to the patients in that these hematopoietic progenitor cells may survive longer, and divide to yield a broader range of gene-corrected cells. (Protocol #9007-002)

Appendix D-16. Dr. Steven A. Rosenberg of the National Institutes of Health, Bethesda, Maryland, can conduct experiments on patients with advanced melanoma who have failed all effective therapy. These patients will be treated with escalating doses of autologous tumor infiltrating lymphocytes (TIL) transduced with a gene coding for tumor necrosis factor (TNF). Escalating numbers of transduced TIL will be administered at three weekly intervals along with the administration of interleukin-2 (IL-2). The objective is to evaluate the toxicity and possible therapeutic efficacy of the administration of tumor infiltrating lymphocytes (TIL) transduced with the gene coding for TNF. (Protocol #9007-003)

Appendix D-17. Dr. Malcolm K. Brenner of St. Jude Children's Research Hospital of Memphis, Tennessee, can conduct experiments on patients with acute myelogenous leukemia (AML). Using the LNL6 retroviral vector, the autologous bone marrow cells will be transduced with the gene coding for neomycin resistance. The purpose of this gene marking experiment is to determine whether the source of relapse after autologous bone marrow transplantation for acute myelogenous leukemia is residual malignant cells in the harvested marrow or reoccurrence of tumor in the patient. Determining the source of relapse should indicate whether or not purging of the bone marrow is a necessary procedure. (Protocol #9102-004)

Appendix D-18. Dr. Malcolm K. Brenner of St. Jude Children's Research Hospital of Memphis, Tennessee, can conduct experiments on pediatric patients with Stage D (disseminated) neuroblastoma who are being treated with high-dose carboplatin and etoposide in either phase I/II or phase II trials. All the patients in these studies will be subjected to bone marrow transplantation since it will allow them to be exposed to chemoradiation that would be lethal were it not for the availability of stored autologous marrow for rescue. The bone marrow cells of these patients will be transduced with the gene coding for neomycin resistance using the LNL6 vector. The purpose of this gene marking study is to determine whether the source of relapse after autologous bone marrow transplantation is residual malignant cells in the harvested marrow or residual disease in the patient. Secondly,

it is hoped to determine the contribution of marrow autographs to autologous reconstitution. (Protocol #9105-005/9105-006)

Appendix D-19. Dr. Albert B. Deisseroth of the MD Anderson Cancer Center of Houston, Texas, can conduct experiments on patients with chronic myelogenous leukemia who have been reinduced into a second chronic phase or blast cells. The patients in these studies will receive autologous bone marrow transplantation. Using the LNL6 vector, the bone marrow cells will be transduced with the gene coding for neomycin resistance. The purpose of these marking studies is to determine if the origin of relapse arises from residual leukemic cells in the patients or from viable leukemic cells remaining in the bone marrow used for autologous transplantation. (Protocol #9105-007)

Appendix D-20. Drs. Fred D. Ledley and Savio L. C. Woo of Baylor College of Medicine of Houston, Texas, can conduct experiments on pediatric patients with acute hepatic failure who are identified as candidates for hepatocellular transplantation. Using the LNL6 vector, the hepatocytes will be transduced with the gene coding for neomycin resistance. The purpose of using a genetic marker is to demonstrate the pattern of engraftment of transplanted hepatocytes and to help determine the success or failure of engraftment. (Protocol #9105-008)

Appendix D-21. Dr. Steven A. Rosenberg of the National Institutes of Health, Bethesda, Maryland, can conduct experiments on patients with advanced melanoma, renal cell cancer, and colon carcinoma who have failed all effective therapy. In an attempt to increase these patients' immune responses to the tumor, the tumor necrosis factor gene or the interleukin-2 gene will be introduced into a tumor cell line established from the patient. These gene-modified autologous tumor cells will then be injected into the thigh of the patient. To further utilize the immune system of the patient to fight the tumor, stimulated lymphocytes will be cultured from either the draining regional lymph nodes or the injected tumor itself. The patients will be evaluated for antitumor effects engendered by the injection of the gene modified tumor cells themselves as well as after the infusion of the cultured lymphocytes. (Protocol #9110-010/9110-011)

Appendix D-22. Dr. James M. Wilson of the University of Michigan Medical Center of Ann Arbor, Michigan, can conduct experiments on three patients with the homozygous form of familial hypercholesterolemia. Both children and adults will be eligible for this therapy. In an attempt to correct the basic genetic defect in this disease, the gene coding for the low-density lipoprotein (LDL) receptor will be introduced into liver cells taken from the patient. The gene-corrected hepatocytes will then be infused into the portal circulation of the patient through an indwelling catheter. The patients will be evaluated for engraftment of these treated hepatocytes through a series of metabolic studies; three months after gene therapy, a liver biopsy will be taken and analyzed for the presence of recombinant derived RNA and DNA to document the presence of the gene coding for the normal LDL receptor. (Protocol #9110-012)

Appendix D-23. Dr. Michael T. Lotze of the University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, can conduct experiments on 20 patients with metastatic melanoma who have failed conventional therapy. A gene transfer experiment will be performed, transducing the patients' tumor infiltrating lymphocytes (TILs) with the gene for neomycin resistance. Through the use of this gene marking technique, it is proposed to determine how long TIL cells can be detected *in vivo* in the peripheral blood of the patients, and how the administration of interleukin-2 and interleukin-4 affects localization and survival of TIL cells in tumor sites. (Protocol #9105-009)

Appendix D-24. Dr. Gary J. Nabel of the University of Michigan Medical School, Ann Arbor, Michigan, can conduct gene therapy experiments on twelve patients with melanoma or adenocarcinoma. Patient population will be limited to adults over the age of 18 and female patients must be postmenopausal or have undergone tubal ligation or orchiectomy. The patient's immune response will be stimulated by the introduction of a gene encoding for a Class I MHC protein, HLA-B7, in order to enhance tumor regression. DNA/liposome-mediated transfection techniques will be used to directly transfer this foreign gene into tumor cells. HLA-B7 expression will be confirmed *in vivo*, and the immune response stimulated by the expression of this antigen will be characterized. These experiments will be analyzed for their efficacy in treating cancer. (Protocol #9202-013)

Appendix D-25. Kenneth Cornetta of Indiana University, Indianapolis, Indiana, can conduct gene transfer experiments on up to 10 patients with acute myelogenous leukemia (AML) and up to 10 patients with acute lymphocytic leukemia (ALL). The patient population will be limited to persons between 18 and 65 years of age. Using the LNL-6 vector, autologous bone marrow cells will be marked with the neomycin resistance gene. Gene marked and untreated bone marrow cells will be reinfused at the time of bone marrow transplantation. Patients

will then be monitored for evidence of the neomycin resistance gene in peripheral blood and bone marrow cells in order to determine whether relapse of their disease is a result of residual malignant cells remaining in the harvested marrow or inadequate ablation of the tumor cells by chemotherapeutic agents. Determining the source of relapse may indicate whether or not purging of the bone marrow is a necessary procedure for these leukemia patients. Further studies will be performed in order to determine the percentage of leukemic cells that contain the LNL-6 vector and the clonality of the marked cells. (Protocol #9202-014)

Appendix D-26. Dr. James S. Economou of the University of California, Los Angeles, can conduct gene transfer experiments on 20 patients with metastatic melanoma and 20 patients with renal cell carcinoma. These patients will be treated with various combinations of tumor-infiltrating lymphocytes and peripheral blood leukocytes, including CD8 and CD4 subsets of both types of cells. These effector cell populations will be given in combination with interleukin-2 (IL-2) in the melanoma patients and IL-2 plus alpha interferon in the renal cell carcinoma patients. The effector cells will be transduced with the neomycin resistance gene using either the LNL6 or G1N retroviral vectors. This "genetic marking" of the tumor-infiltrating lymphocytes and peripheral blood lymphocytes is designed to answer questions about the trafficking of these cells, their localization to tumors, and their *in vivo* life span. (Protocol #9202-015)

Appendix D-27. Drs. Philip Greenberg and Stanley R. Riddell of the Fred Hutchinson Cancer Research Center, Seattle, Washington, may conduct gene transfer experiments on 15 human immunodeficiency virus (HIV) seropositive patients (18-45 years old) undergoing allogeneic bone marrow transplantation for non-Hodgkin's lymphoma and 15 HIV-seropositive patients (18-50 years old) who do not have acquired immunodeficiency syndrome (AIDS)-related lymphoma and who are not undergoing bone marrow transplantation to evaluate the safety and efficacy of HIV-specific cytotoxic T lymphocyte (CTL) therapy. CTL will be transduced with a retroviral vector (HyTK) encoding a gene that is a fusion product of the hygromycin phosphotransferase gene (HPH) and the herpes simplex virus thymidine kinase (HSV-TK) gene. This vector will deliver both a marker gene and an ablatable gene in these T cell clones in the event that patients develop side effects as a consequence of CTL therapy. Data will be correlated over time, looking at multiple parameters of HIV disease activity. The objectives of these studies include evaluating the safety and toxicity of CTL therapy, determining the duration of *in vivo* survival of HIV-specific CTL clones, and determining if ganciclovir therapy can eradicate genetically modified, adoptively transferred CTL cells. (Protocol #9202-017)

Appendix D-28. Dr. Malcolm Brenner of St. Jude Children's Research Hospital, Memphis, Tennessee, can conduct gene therapy experiments on twelve patients with relapsed/refractory neuroblastoma who have relapsed after receiving autologous bone marrow transplant. In an attempt to stimulate the patient's immune response, the gene coding for Interleukin-2 (IL-2) will be used to transduce tumor cells, and these gene-modified cells will be injected subcutaneously in a Phase 1 dose escalation trial. Patients will be evaluated for an anti-tumor response. (Protocol #9206-018)

Appendix D-29. Drs. Edward Oldfield, Kenneth Culver, Zvi Ram, and R. Michael Blaese of the National Institutes of Health, Bethesda, Maryland, can conduct gene therapy experiments on ten patients with primary malignant brain tumors and ten patients with lung cancer, breast cancer, malignant melanoma, or renal cell carcinoma who have brain metastases. The patient population will be limited to adults over the age of 18. Patients will be divided into two groups based on the surgical accessibility of their lesions. Both surgically accessible and surgically inaccessible lesions will receive intra-tumoral injections of the retroviral Herpes simplex thymidine kinase (HS-tk) vector-producer cell line, G1TkSvNa, using a guided stereotaxic approach. Surgically accessible lesions will be excised seven days after stereotaxic injection, and the tumor bed will be infiltrated with the HS-tk producer cells. The removed tumor will be evaluated for the efficiency of transduction. Ganciclovir (GCV) will be administered beginning on the fifth postoperative day. In the case of surgically inaccessible lesions, the patients will receive intravenous therapy with GCV seven days after receiving the intra-tumoral injections of the retroviral HS-tk vector-producer cells. (Protocol #9206-019)

Appendix D-30. Dr. Albert D. Deisseroth of MD Anderson Cancer Center, Houston, Texas, can conduct gene transfer experiments on ten patients who have developed blast crisis or accelerated phase chronic myelogenous leukemia (CML). The retroviral vectors G1N and LNL6 which code for neomycin resistance will be used to transduce autologous peripheral blood and bone marrow cells that have been removed and stored at the time of cytogenetic remission or re-induction of chronic phase in Philadelphia chromosome positive CML patients. Following reinduction of the chronic phase of CML and preparative chemotherapy, patients will be infused with the transduced autologous cells.

This protocol is designed to determine the cause of relapse of CML. If polyclonal CML neomycin marked blastic cells appear at the time of relapse, their presence will indicate that relapse arises from the leukemic CML blast cells present in the autologous cells infused following chemotherapy. If residual systemic disease contributes to relapse, the neomycin resistance gene will not be detected in the CML leukemic blasts at the time of relapse.

This study will compare the relative contributions of the peripheral blood and bone marrow to generate hematopoietic recovery after bone marrow transplantation and evaluate purging and selection of peripheral blood or bone marrow as a source of stem cells for transplant. The percentage of neomycin resistant CML cells which are leukemic will be determined by PCR analysis and detection of bcr-abl mRNA. (Protocol #9206-020)

Appendix D-31. Dr. Cynthia Dunbar of the National Institutes of Health, Bethesda, Maryland, can conduct gene transfer experiments on up to 48 patients with multiple myeloma, breast cancer, or chronic myelogenous leukemia. The retroviral vectors G1N and LNL6 will be used to transfer the neomycin resistance marker gene into autologous bone marrow and peripheral blood stem cells in the presence of growth factors to examine hematopoietic reconstitution after bone marrow transplantation. The efficiency of transduction of both short and long term autologous bone marrow reconstituting cells will be examined.

Autologous bone marrow and CD34+ peripheral blood stem cells will be enriched prior to transduction. Myeloma and CML patients will receive both autologous bone marrow and peripheral blood stem cell transplantation. These separate populations will be marked with both the G1N and LNL6 retroviral vectors. If short and long term marking experiments are successful, important information may be obtained regarding the biology of autologous reconstitution, the feasibility of retroviral gene transfer into hematopoietic cells, and the contribution of viable tumor cells within the autograft to disease relapse. (Protocol #9206-023/9206-024/9206-025)

Appendix D-32. Dr. Bernd Gansbacher of the Memorial Sloan-Kettering Cancer Center, New York, New York, can conduct gene therapy experiments on twelve patients over 18 years of age with metastatic melanoma who are HLA-A2 positive and who have failed conventional therapy. This is a phase I study to examine whether allogeneic HLA-A2 matched melanoma cells expressing recombinant human Interleukin-2 (IL-2) can be injected subcutaneously and used to create a potent tumor specific immune response without producing toxicity. By allowing the tumor cells to present the MHC Class I molecule as well as the secreted IL-2, a clonal expansion of tumor specific effector cells is expected. These effector populations may access residual tumor at distant sites via the systemic circulation. (Protocol #9206-021)

Appendix D-33. Dr. Bernd Gansbacher of the Memorial Sloan-Kettering Cancer Center, New York, New York, can conduct gene therapy experiments on twelve patients over 18 years of age with renal cell carcinoma who are HLA-A2 positive and who have failed conventional therapy. This Phase I study will examine whether allogeneic HLA-A2 matched renal cell carcinoma cells expressing recombinant human Interleukin-2 (IL-2) can be injected subcutaneously and used to create a potent tumor specific immune response without producing toxicity. By allowing the tumor cells to present the MHC Class I molecule as well as the secreted IL-2, a clonal expansion of tumor specific effector cells is expected. These effector populations may access residual tumor at distant sites via the systemic circulation. (Protocol #9206-022)

Appendix D-34. Dr. Michael T. Lotze, University of Pittsburgh, Pittsburgh, Pennsylvania, can conduct experiments on twenty patients with metastatic, and/or unresectable, locally advanced melanoma, renal cell carcinoma, breast cancer, or colon cancer who have failed standard therapy. Patients will receive multiple subcutaneous injections of autologous tumor cells combined with an autologous fibroblast cell line that has been transduced *in vitro* with the gene coding for Interleukin-4 (IL-4) to augment the *in vivo* antitumor effect. Patients will be monitored for antitumor effect by PCR analysis and multiple biopsy of the injection site. (Protocol #9209-033)

Appendix D-35. Dr. Friedrich G. Schuening, Fred Hutchinson Cancer Research Center, Seattle, Washington, can conduct human gene transfer experiments on patients \geq 18 years of age with breast cancer, Hodgkin's disease, or non-Hodgkin's lymphoma. A total of 10 patients per year will be enrolled in the studies over a period of four years. Patients will undergo autologous bone marrow transplantation with a selected population of Interleukin-3 (IL-3) or granulocyte colony-stimulating factor (G-CSF) stimulated CD34(+) peripheral blood repopulating cells (PBRC) that have been transduced with the gene coding for neomycin resistance (neo^R) using the retroviral vector, LN. Patients will be continuously monitored for neo^R to determine the relative contribution

of autologous PBRCs to long-term hematopoietic reconstitution. Demonstration of long-term contribution of autologous PBRC to hematopoiesis will enable the use of PBRC alone for autologous transplants and suggest the use of PBRC as long-term carriers of therapeutically relevant genes. (Protocol #9209-027/9209-028)

Appendix D-36. Dr. Friedrich G. Schuening, Fred Hutchinson Cancer Research Center, Seattle, Washington, can conduct human gene transfer experiments on patients ≥ 18 years of age with breast cancer, Hodgkin's disease, or non-Hodgkin's lymphoma. A total of 5 patients per year will be enrolled in the study over a period of four years. Patients will undergo allogeneic bone marrow transplant with granulocyte colony-stimulating factor (G-CSF) stimulated CD34(+) PBRC harvested from an identical twin that have been transduced with neo^R using the retroviral vector, LN. Patients will be continuously monitored for neo^R to determine the relative contribution of G-CSF stimulated allogeneic PBRCs to long-term bone marrow engraftment. Demonstration of long-term contribution of allogeneic PBRC to hematopoiesis will enable the use of PBRC alone for allogeneic transplants and suggest the use of PBRC as long-term carriers of therapeutically relevant genes. (Protocol #9209-029)

Appendix D-37. Dr. Malcolm K. Brenner of St. Jude Children's Hospital, Memphis, Tennessee, and Dr. Bonnie J. Mills of Baxter Healthcare Corporation, Santa Ana, California, can conduct a multicenter uncontrolled human gene transfer experiment on 12 patients ≤ 21 years of age with Stage D Neuroblastoma in first or second marrow remission. Autologous bone marrow cells will be separated into two fractions, purged and unpurged. Each fraction will be transduced with the neo^R gene by either LNL6 or G1Na. Patients will be monitored by the polymerase chain reaction (PCR) for the presence of neo^R. The protocol is designed to evaluate the safety and efficacy of the Neuroblastoma Bone Marrow Purging System following high dose chemotherapy. (Protocol #9209-032)

Appendix D-38. Drs. Carolyn Keierleber and Ann Progulske-Fox of the University of Florida, Gainesville, Florida, can conduct experiments involving the introduction of a gene coding for tetracycline resistance into *Porphyromonas gingivalis* at a physical containment level of Biosafety Level-2 (BL-2).

Appendix D-39. Dr. Scott M. Freeman of Tulane University Medical Center, New Orleans, Louisiana, can conduct experiments on patients with epithelial ovarian carcinoma who have clinical evidence of recurrent, progressive, or residual disease who have no other therapy available to prolong survival. Patients will be injected intraperitoneally with the irradiated PA-1 ovarian carcinoma cell line which has been transduced with the herpes simplex thymidine kinase (HSV-TK) gene. The patients will then receive ganciclovir therapy. Previous, data indicates that HSV-TK+ tumor cells exhibit a killing effect on HSV-TK- cells when exposed to ganciclovir therapy. Patients will be evaluated for safety and side effects of this treatment. (Protocol #9206-016)

Appendix D-40. Dr. Michael J. Welsh, Howard Hughes Medical Institute Research Laboratories, University of Iowa College of Medicine, Iowa City, Iowa, may conduct experiments on 3 cystic fibrosis (CF) patients ≥ 18 years of age with mild to moderate disease. This Phase I study will determine the: (1) *in vivo* safety and efficacy of the administration of the replication-deficient type 2 adenovirus vector, Ad2/CFTR-1, to the nasal epithelium; (2) efficacy in correcting the CF chloride transport defect *in vivo*; and (3) effect of adenovirus vector dosage on safety and efficacy. (Protocol #9212-036)

Appendix D-41. Dr. Ronald G. Crystal, National Institutes of Health, Bethesda, Maryland, may conduct experiments on 10 cystic fibrosis (CF) patients ≥ 21 years of age. Patients will receive an initial administration of the replication-deficient type 5 adenovirus vector, AdCFTR, to their left nares. If no toxicity is observed from intranasal administration, patients will receive a single administration of AdCFTR to the respiratory epithelium of their left large bronchi. Five groups of patients (2 patients per group) will be studied based on increased dosage administration of AdCFTR. This study will determine the: (1) *in vivo* safety and efficacy of the administration of AdCFTR into the respiratory epithelium; (2) efficacy of the correction of the biologic abnormalities of CF in the respiratory epithelium; (3) duration of the biologic correction; (4) efficacy of the correction of the abnormal electrical potential difference of the airway epithelial sheet; (5) clinical parameters relevant to the disease process; and (6) if humoral immunity develops against AdCFTR sufficient to prevent repeat administration. (Protocol #9212-034)

Appendix D-42. Dr. Kenneth Culver, Iowa Methodist Medical Center, Des Moines, Iowa, and Dr. John Van Gilder, University of Iowa, Iowa City, Iowa, may conduct experiments on 15 patients ≥ 18 years of age with recurrent malignant primary brain tumors or lung, melanoma, renal cell carcinoma, or breast carcinoma brain metastases who have failed standard therapy for their disease. Patient eligibility will be limited to those patients

who have measurable residual tumor immediately following the post-operative procedure as demonstrated by imaging studies. The number of patients treated will be equally divided between the Iowa Methodist Medical Center and the University of Iowa. If a positive response is observed in any of the first 15 patients, the investigators may submit a request to treat an additional 15 patients.

Following surgical debulking, patients will receive a maximum of 3 intralesional injections of the G1TkSvNa vector-producing cell line (VPC) to induce regression of residual tumor cells by ganciclovir (GCV) therapy. Patients who demonstrate stable disease for a minimum of 6 months following this treatment will be eligible for additional VPC injections and subsequent GCV therapy. (Protocol #9303-037)

Appendix D-43. Drs. Malcolm Brenner, Robert Krance, Helen E. Heslop, Victor Santana, and James Ihle, St. Jude Children's Research Hospital, Memphis, Tennessee, may conduct experiments on 35 patients ≥ 1 year and ≤ 21 years of age at the time of initial diagnosis of acute myelogenous leukemia (AML). The investigators will use the two retroviral vectors, LNL6 and G1Na, to determine the efficacy of the bone marrow purging techniques: 4-hydroxyperoxycyclophosphamide and interleukin-2 (IL-2) activation of endogenous cytotoxic effector cells, in preventing relapse from the reinfusion of autologous bone marrow cells. (Protocol #9303-039)

Appendix D-44. Drs. Helen E. Heslop, Malcolm Brenner, and Cliona Rooney, St. Jude Children's Research Hospital, Memphis, Tennessee, may conduct experiments of 35 patients ≤ 21 years of age who will be recipients of mismatched-related or phenotypically similar unrelated donor marrow grafts for leukemia. In this Phase I dose escalation study, spontaneous lymphoblastoid cell lines will be established that express the same range of Epstein-Barr Virus (EBV) encoded proteins as the recipient. These EBV-specific cell lines will be transduced with LNL6 or G1Na and readministered at the time of bone marrow transplant. This study will determine: (1) survival and expansion of these EBV-specific cell lines *in vivo*, (2) the ability of these adoptively transferred cells to confer protection against EBV infection, and (3) appropriate dosage and administration schedules. (Protocol #9303-038)

Appendix D-45. Drs. Robert W. Wilmott and Jeffrey Whitsett, Children's Hospital Medical Center, Cincinnati, Ohio, and Dr. Bruce Trapnell, Genetic Therapy, Inc., Gaithersburg, Maryland, may conduct experiments on 15 cystic fibrosis (CF) patients who have mild to moderate disease ≥ 21 years of age. The replication-deficient type 5 adenovirus vector, Av1CF2, will be administered to the nasal and lobar bronchial respiratory tract of patients. This study will demonstrate the: (1) expression of normal cystic fibrosis transmembrane conductance regulator (CFTR) mRNA *in vivo*, (2) synthesis of CFTR protein, and (3) correction of epithelial cell cAMP dependent Cl⁻ permeability. The pharmacokinetics of CFTR expression and ability to re-infect the respiratory tract with AvCF2 will be determined. Systemic and local immunologic consequences of Av1CF2 infection, the time of viral survival, and potential for recombination or complementation of the virus will be monitored. (Protocol #9303-041)

Appendix D-46. Dr. James M. Wilson of the University of Pennsylvania Medical Center, Philadelphia, Pennsylvania, may conduct experiments on 20 adult patients with advanced cystic fibrosis lung disease. An isolated segment of the patients' lung will be transduced with the E1 deleted, replication-incompetent adenovirus vector, Ad.CB-CFTR using a bronchoscope for gene delivery. Ad.CB-CFTR contains the human gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein. Pulmonary biopsies will be obtained by bronchoscopy at 4 days, 6 weeks, and 3 months following treatment. Patients will be monitored for evidence of CFTR gene transfer and expression, immunological responses to CFTR or adenovirus proteins, and toxicity. (Protocol #9212-035)

Appendix D-47. Dr. Hilliard F. Seigler of Duke University Medical Center, Durham, North Carolina, may conduct experiments on 20 patients with disseminated malignant melanoma. Autologous tumor cells will be transduced with a retroviral vector, pHuy-IFN, that contains the gene encoding human γ -IFN. Following lethal irradiation, the transduced cells will be readministered to patients for the purpose of generating cytotoxic T cells that are tumor specific along with the up-regulation of Class I major histocompatibility antigens. Patients will be monitored for clinical regression of tumors and generation of tumor-specific cytotoxic T lymphocytes. (Protocol #9306-043)

Appendix D-48. Drs. Stefan Karlsson and Cynthia Dunbar of the National Institutes of Health, Bethesda, Maryland, and Dr. Donald B. Kohn of the Children's Hospital of Los Angeles, Los Angeles, California, may conduct experiments on 10 patients with Gaucher disease. CD34(+) hematopoietic stem cells will be isolated from bone marrow or from peripheral blood treated with granulocyte-colony stimulating factor. CD34(+) cells will

be transduced with a retrovirus vector, G1Gc, containing cDNA encoding human glucocerebrosidase and administered intravenously. Patients will be monitored for toxicity and glucocerebrosidase expression. (Protocol #9306-047)

Appendix D-49. Dr. Gary J. Nabel of the University of Michigan Medical Center, Ann Arbor, Michigan, may conduct experiments on 12 patients with AIDS to be divided into 4 experimental groups. CD4(+) lymphocytes will be isolated from peripheral blood and transduced with Rev M10, a transdominant inhibitory mutant of the *rev* gene of the human immunodeficiency virus (HIV). Transduction of the *rev* mutant will be mediated either by the retrovirus vector, PLJ-cREV M10, or by particle-mediated gene transfer of plasmid DNA. Patients will be monitored for survival of the transduced CD4(+) cells by polymerase chain reaction and whether Rev M10 can confer protection against HIV infection to CD4(+) cells. (Protocol #9306-049)

Appendix D-50. Dr. Gary J. Nabel of the University of Michigan Medical Center, Ann Arbor, Michigan, may conduct experiments on 24 patients with advanced cancer. Patients will undergo *in vivo* transduction with DNA/liposome complexes containing genes encoding the HLA-B7 histocompatibility antigen and beta-2 microglobulin in a non-viral plasmid. These DNA/liposome complexes will be administered either by intratumoral injection or catheter delivery. Patients will be monitored for enhanced immune responses against tumor cells, and safe and effective doses will be determined. (Protocol #9306-045)

Appendix D-51. Dr. John A. Barranger of the University of Pittsburgh, Pittsburgh, Pennsylvania, may conduct experiments on 5 patients with Gaucher disease. The CD34(+) hematopoietic stem cells will be isolated from peripheral blood and transduced *in vitro* with the retrovirus vector, N2-Sv-GC, encoding the glucocerebrosidase (GC) enzyme. Following reinfusion of the transduced cells, patients will be monitored by PCR analysis for GC expression in peripheral blood leukocytes. Patients currently receiving GC replacement therapy and who demonstrate clinical responsiveness will be withdrawn from exogenous GC therapy. Patients not previously treated with exogenous GC, will be monitored for clinical reversal of lipid storage symptoms. (Protocol #9306-046)

Appendix D-52. Dr. Robert Walker of the National Institutes of Health, Bethesda, Maryland, may conduct experiments on 12 HIV-infected patients who have a seronegative identical twin. CD4(+) and CD8(+) cells will be isolated from the seronegative twin and induced to polyclonal proliferation with anti-CD3 and interleukin-2. The enriched population of cells will be transduced with either LNL6 or G1Na, which contain the neo^R gene. The transduced cells will be expanded in tissue culture and administered to the HIV-infected twin. Patients will be monitored for immune function and the presence of marked cells. (Protocol #9209-026)

Appendix D-53. Dr. Corey Raffel of the Children's Hospital Los Angeles, Los Angeles, California, and Dr. Kenneth Culver of Iowa Methodist Medical Center, Des Moines, Iowa, may conduct experiments on 30 patients between 2 and 18 years of age with recurrent malignant astrocytoma. Fifteen patients will be accrued into this study initially. If at least one patient responds to therapy, an additional 14 patients will be treated. Patients with either surgically accessible or non-accessible tumors will be treated with the vector producing cell line (PA317) carrying the retrovirus vector, G1TkSvNa. This vector will transduce tumor cells *in vivo* with the *Herpes simplex* thymidine kinase (HS-tk) gene that renders the cells sensitive to killing by ganciclovir. Surgically accessible patients will undergo surgical debulking of their tumor followed by repeated administration of the HS-tk vector producer cells into the tumor bed. Children with unresectable tumors will undergo stereotaxic injection of vector producer cells into tumors. (Protocol #9306-050)

Appendix D-54. Dr. Jeffrey E. Galpin of the University of Southern California, Los Angeles, California, and Dr. Dennis A. Casciato of the University of California, Los Angeles, California, may conduct experiments on 15 HIV(+) asymptomatic patients. Patients will receive 3 monthly intramuscular injections of the retrovirus vector (N2IIIBenv) encoding the HIV-1 IIIB envelope protein. Patients will be monitored for acute toxicity, CD4 levels, HIV-specific CTL responses, and viral burdens. (Protocol #9306-048)

Appendix D-55. Drs. Charles Hesdorffer and Karen Antman of Columbia University College of Physicians and Surgeons, New York, New York, may conduct experiments on 20 patients with advanced breast, ovary, and brain cancer. CD34(+) hematopoietic stem cells will be isolated from bone marrow, transduced with the retrovirus vector, PHaMDR1/A, and readministered to patients. Patients will be monitored for the presence and expression of the MDR-1 gene. The investigators will determine whether MDR-1 expression increases following chemotherapy. (Protocol #9306-051)

Appendix D-56. Dr. Enzo Paoletti of Virogenetics Corporation, Troy, New York, may conduct experiments with poxvirus vectors NYVAC, ALVAC, and TROVAC at Biosafety Level 1.

Appendix D-57. Drs. Richard C. Boucher and Michael R. Knowles of the University of North Carolina, Chapel Hill, North Carolina, may conduct experiments on 9 patients (18 years old or greater) with cystic fibrosis to test for the safety and efficacy of an E1-deleted recombinant adenovirus containing the cystic fibrosis transmembrane conductance regulator (CFTR) cDNA, Ad.CB-CFTR. A single dose of 10^8 , 3×10^9 or 10^{11} pfu/ml will be administered to the nasal cavity of 3 patients in each dose group. Patients will be monitored by nasal lavage and biopsy to assess safety and restoration of normal epithelial function. (Protocol #9303-042)

Appendix D-58. Dr. Joyce A. O'Shaughnessy of the National Institutes of Health, Bethesda, Maryland, may conduct experiments on 18 patients (18-60 years old) with Stage IV breast cancer who have achieved a partial or complete response to induction chemotherapy. This study will determine the feasibility of obtaining engraftment of CD34(+) hematopoietic stem cells transduced by a retroviral vector, G1MD, and expressing a cDNA for the human multi-drug resistance-1 (MDR-1) gene following high dose chemotherapy, and whether the transduced MDR-1 gene confers drug resistance to hematopoietic cells and functions as an *in vivo* dominant selectable marker. Patients will be monitored for evidence of myeloprotection and presence of the transduced MDR-1 gene." (Protocol #9309-054)

Appendix D-59. Drs. Larry E. Kun, R. A. Sanford, Malcolm Brenner, and Richard L. Heideman of St. Jude Children's Research Hospital, Memphis, Tennessee, and Dr. Edward H. Oldfield of the National Institutes of Health, Bethesda, Maryland, may conduct experiments on 6 patients (3-21 years old) with progressive or recurrent malignant supratentorial tumors resistant to standard therapies. Mouse cells producing the retroviral vector containing the herpes simplex thymidine kinase gene (G1TKSVNa) will be instilled into the tumor areas via multiple stereotactically placed cannulas. Patients will be treated with ganciclovir to eliminate cells expressing the transduced gene. Patients will be monitored for central nervous system, hematologic, renal or other toxicities, and for anti-tumor responses by magnetic resonance imaging studies. (Protocol #9309-055)

Appendix D-60. The physical containment level may be reduced from Biosafety Level 3 to Biosafety Level 2 for a Semliki Forest Virus (SFV) vector expression system of Life Technologies, Inc., Gaithersburg, Maryland.

Appendix D-61. Dr. Albert B. Deisseroth of the University of Texas MD Anderson Cancer Center, Houston, Texas, may conduct experiments on 10 patients (≥ 16 to ≤ 60 years of age) with chronic lymphocytic leukemia. Autologous peripheral blood and bone marrow cells will be removed from patients following chemotherapy and marked by transduction with two distinguishable retroviral vectors, G1Na and LNL6, containing the neomycin resistance gene. The gene marked cells will be reinfused into patients to determine the efficiency of bone marrow purging and the origin of relapse following autologous bone marrow transplantation. (Protocol #9209-030)

Appendix D-62. Dr. Jonathan Simons of the Johns Hopkins Oncology Center, Baltimore, Maryland, may conduct experiments on 26 patients (≥ 18 years of age) with metastatic renal cell carcinoma to evaluate the safety and tolerability of intradermally injected autologous irradiated tumor cells transduced with the retrovirus vector, MFG, containing the human granulocyte-macrophage colony stimulating factor gene. Acute and long-term clinical toxicities and *in vitro* and *in vivo* induction of specific anti-tumor immune responses will be monitored. (Protocol #9303-040)

Appendix D-63. Dr. Albert B. Deisseroth of the University of Texas MD Anderson Cancer Center, Houston, Texas, may conduct experiments on 20 patients (≥ 18 and ≤ 60 years old) with ovarian cancer. A murine viral vector was constructed from the third generation of L series retroviruses with the insert of the human multi-drug resistance-1 (MDR-1) transduced gene. The investigators will assess the safety and feasibility of administering CD34 (+) autologous peripheral blood and bone marrow cells. Patients will be monitored for the presence of the MDR-1 gene and for the effect of gene transfer on hematopoietic function following the transplantation. (Protocol #9306-044)

Appendix D-64. Dr. Joseph Ilan of the Case Western Reserve University School of Medicine and University Hospital of Cleveland, Cleveland, Ohio, may conduct experiments on 12 patients (≥ 18 years of age) with advanced brain cancer. Human malignant glioma tumor cells will be cultured, transfected with Epstein-Barr virus (EBV)-based vector, anti-Insulin growth factor-I, lethally irradiated, and injected subcutaneously into

patients in an attempt to express antisense Insulin growth factor-1. Patients will be monitored for toxicity and immunologic responses to the vector. (Protocol #9306-052)

Appendix D-65. Drs. James S. Economou and John Glaspy of the University of California, Los Angeles, California, may conduct experiments on 30 patients (≥ 18 to ≤ 70 years of age) with metastatic melanoma. A human melanoma cell line (M-24) will be transduced with the retroviral vector, G1NaCvi2, expressing the human interleukin-2 (IL-2) gene. The IL-2 producing cells will be mixed with the patient's autologous tumor cells, irradiated, and injected subcutaneously in an attempt to enhance the tumor-specific immunologic response. Patients will be monitored for toxicity, *in vitro* and *in vivo* immunologic responses, and clinical anti-tumor effects. (Protocol #9309-058)

Appendix D-66. Drs. Peter Cassileth, Eckhard R. Podack, and Kasi Sridhar of the University of Miami, and Niramol Savaraj of the Miami Veterans Administration Hospital, Miami, Florida, may conduct experiments on 12 patients (≥ 18 years of age) with limited stage small cell lung cancer. Autologous tumor cells will be removed, expanded in culture, and transduced by lipofection with the BMG-Neo-hIL2 vector (derived from bovine papilloma virus). The objective of this protocol is to demonstrate the safety and efficacy of administering IL-2 transduced autologous tumor cells in an attempt to stimulate a tumor-specific cytotoxic T lymphocyte (CTL) response, and to determine the quantity and characteristics of the CTL that have been generated. (Protocol #9309-053)

Appendix D-67. Drs. Edward H. Oldfield and Zvi Ram of the National Institutes of Health, Bethesda, Maryland, may conduct experiments on 20 patients (≥ 18 years of age) with leptomeningeal carcinomatosis. The patients will receive intraventricular or subarachnoid injection of murine vector producing cells containing the retroviral vector, G1Tk1SvNa. Tumor cells expressing the herpes simplex thymidine kinase gene will be rendered sensitive to killing by subsequent administration of ganciclovir. Patients will be monitored for safety and anti-tumor response by magnetic resonance imaging (MRI) and cerebral spinal fluid cytological analysis. (Protocol #9312-059)

Appendix D-68. Drs. Tapas K. Das Gupta and Edward P. Cohen of the University of Illinois College of Medicine, Chicago, Illinois, may conduct experiments on 12 subjects who differ in at least 3 out of 6 alleles at the Class I histocompatibility locus (≥ 18 years of age) with Stage IV malignant melanoma. The subjects will be immunized with a lethally irradiated allogeneic human melanoma cell line transduced with the human interleukin-2 expressing retroviral vector, pZipNeoSv-IL-2. Subjects will be monitored for toxicity, induction of B and T cell antitumor responses *in vitro* and *in vivo*, and any clinical evidence of antitumor effect. (Protocol #9309-056)

Appendix D-69A. Dr. Michael J. Welsh of the Howard Hughes Medical Institute, Iowa City, Iowa, may conduct experiments on 20 patients (≥ 18 years of age) with cystic fibrosis. The investigator will administer increasing doses of either of the two adenovirus vectors, AD2/CFTR-1 or AD2-ORF6/PGK-CFTR, to the nasal epithelium of 10 patients (1 nostril) or maxillary sinus epithelium of 10 patients (1 maxillary sinus). Patients will be isolated for a period of 24 hours following vector administration; however, if 1 patient demonstrates secreted virus at 24 hours, the investigator will notify the Recombinant DNA Advisory Committee for reconsideration of the isolated period. Patients will be assessed for the safety and efficacy of multiply administration of adenovirus vectors encoding the cystic fibrosis transmembrane conductance regulator (CFTR) gene. (Protocol #9312-067)

Appendix D-69B. Dr. Richard Haubrich of the University of California at San Diego Treatment Center, San Diego, California, may conduct experiments on 25 human immunodeficiency virus (HIV)-infected, seropositive, asymptomatic subjects (≥ 18 to ≤ 65 years of age). Subjects will receive 3 monthly intramuscular injections of the retroviral vector, N2/IIIB *env/rev*, which encodes for HIV-1 IIIB *env/rev* proteins. The objective of the study is to induce HIV-1- specific CD8(+) cytotoxic T lymphocyte and antibody responses in order to eliminate HIV-infected cells and residual virus. This Phase I/II study will evaluate acute toxicity, identify long-term treatment effects, and evaluate the disease progression. (Protocol #9312-062)

Appendix D-70. Dr. Mario Sznol of the National Institutes of Health, Frederick, Maryland, may conduct experiments on 50 subjects (≥ 18 years of age) with advanced stage melanoma. Subjects will receive subcutaneous injections of lethally irradiated allogeneic melanoma cells that have been transduced by lipofection with the plasmid DNA vector, CMV-B7, derived from bovine papilloma virus to express the human B7 antigen. The B7 antigen, which binds to the CD28 receptor of T cells, will serve as a co-stimulatory signal to elicit an antitumor immune response. Subjects will be monitored for induction of cytotoxic T lymphocyte, antitumor responses *in vitro* and *in vivo* and any clinical evidence of antitumor effect. (Protocol #9312-063)

Appendix D-71. Dr. Joseph Rubin of the Mayo Clinic, Rochester, Minnesota, may conduct experiments on 15 subjects with hepatic metastases from advanced colorectal cancer (≥ 18 years of age). Subjects will receive intratumoral hepatic injections of the plasmid DNA/lipid complex, pHLA-B7/ β -2 microglobulin, expressing a heterodimeric cell surface protein consisting of the HLA-B7 histocompatibility antigen and β -2 microglobulin. Subjects must be HLA-B7 negative. The objective of this study is to determine a safe and effective dose of the DNA/lipid complex. Subjects will be monitored for antigen-specific immune responses and *in vivo* HLA-B7 expression. (Protocol #9312-064)

Appendix D-72. Dr. Nicholas J. Vogelzang of the University of Chicago Medical Center, Chicago, Illinois, may conduct experiments on 15 subjects with metastatic renal cell carcinoma ≥ 18 years of age. Subjects will receive intratumoral injections of the plasmid DNA/liposome vector pHLA-B7/ β -2 microglobulin, expressing a heterodimeric cell surface protein consisting of the HLA-B7 histocompatibility antigen and β -2 microglobulin. Subjects must be HLA-B7 negative. Subjects will be monitored for antigen-specific immune responses and *in vivo* HLA-B7 expression. (Protocol #9403-071)

Appendix D-73. Dr. Evan M. Hersh of the Arizona Cancer Center and Drs. Emmanuel Akporiaye, David Harris, Alison T. Stopeck, Evan C. Unger, and James A. Warneke of the University of Arizona, Tucson, Arizona, may conduct experiments on 15 subjects with metastatic malignant melanoma ≥ 18 years of age. Subjects will receive intratumoral injections of the plasmid DNA/liposome vector, pHLA-B7/ β -2 microglobulin, expressing a heterodimeric cell surface protein consisting of the HLA-B7 histocompatibility antigen and β -2 microglobulin. Subjects must be HLA-B7 negative. Subjects will be monitored for antigen-specific immune responses and *in vivo* HLA-B7 expression. (Protocol #9403-072)

Appendix D-74. Dr. Ralph Freedman of MD Anderson Cancer Center, Houston, Texas, may conduct gene marking experiments on 9 subjects with ovarian carcinoma or peritoneal carcinomatosis (≥ 16 years of age). Autologous CD3(+)/CD8(+) tumor infiltrating lymphocyte derived T cells will be transduced with the retroviral vector G1Na that encodes for neo^R. Subjects will receive intraperitoneal administration of bulk expanded transduced and nontransduced T cells and recombinant interleukin-2. Previously documented tumor sites and normal tissues will be monitored for neo^R and the proportion of CD3(+)/CD8(+) T cells will be determined. (Protocol #9406-075)

Appendix D-75. Drs. Helen Heslop, Malcolm Brenner, and Robert Krance of St. Jude Children's Research Hospital, Memphis, Tennessee, may conduct gene marking experiments on 20 subjects undergoing autologous bone marrow transplantation for therapy of leukemia or solid tumor (< 21 years of age). The distinguishable retroviral vectors, LNL6 and G1Na (both encoding neo^R), will be used to determine the rate of reconstitution of untreated versus cytokine expanded CD34(+) selected autologous bone marrow cells. (Protocol #9406-076)

Appendix D-76. Drs. Albert Deisseroth, Gabriel Hortobagyi, Richard Champlin, and Frankie Holmes of MD Anderson Cancer Center, Houston, Texas, may conduct experiments on 10 fully evaluable subjects (maximum of 20 entered) with Stage III or IV breast cancer (≥ 18 and ≤ 60 years of age). Subjects will receive autologous CD34(+) peripheral blood cells that have been transduced with the retroviral vector, pVMDR-1, which encodes the multi-drug resistance gene. The objective of this study is to evaluate the safety and feasibility of transducing early hematopoietic progenitor cells with pVMDR-1 and to determine *in vivo* selection of chemotherapy resistant hematopoietic cells. (Protocol #9406-077)

Appendix D-77. Drs. Johnson M. Liu and Neal S. Young of the National Institutes of Health, Bethesda, Maryland, may conduct experiments on 6 patients with Fanconi anemia (≥ 5 years of age). Subjects will receive autologous CD34(+) cells that have been transduced with the retroviral vector, FACC, which encodes the normal Fanconi anemia complementation group C gene. The objective of this study is to determine whether autologous FACC transduced hematopoietic progenitor cells can be safely administered to subjects, the extent of engraftment, and correction of cell phenotype. (Protocol #9406-078)

Appendix D-78. Drs. Robert E. Sobol and Ivor Royston of the San Diego Regional Cancer Center, San Diego, California, may conduct experiments on 15 subjects with recurrent residual glioblastoma multi-forme (≥ 18 years of age). Subjects will receive subcutaneous injections of autologous tumor cells that have been lethally irradiated and transduced with the retroviral vector, G1NaCvi2.23, which encodes for interleukin-2. Subjects will be monitored *in vitro* for cellular and humoral antitumor responses and *in vivo* for antitumor activity. (Protocol #9406-080)

Appendix D-79. Dr. Alfred E. Chang of the University of Michigan Medical Center, Ann Arbor Michigan, may conduct gene marking experiments on 15 subjects with metastatic melanoma (≥ 18 years of age). Subjects will undergo adoptive immunotherapy of anti-CD3/interleukin-2 activated lymph node cells that have been primed *in vivo* with tumor cells that have been transduced with the retrovirus vector, GBAH4, encoding the gene for interleukin-4. The investigator will evaluate the antitumor efficacy and *in vivo* immunological reactivity in subjects receiving adoptively transferred T cells, and the *in vitro* immunological reactivities of the activated T cells that might correlate with their *in vivo* antitumor function. (Protocol #9312-065)

Appendix D-80. Dr. Robert Walker of the National Institutes of Health, Bethesda, Maryland, may conduct gene marking experiments on 40 HIV(+) subjects (≥ 18 years of age). The investigator may also enter an additional number of subjects (to be determined by the investigator) who will receive a single administration of 1×10^7 HIV-specific CD8(+) cells. The investigator will: (1) Assess the safety and tolerance of the adoptive transfer of anti-HIV cytotoxic, syngeneic, CD8(+) peripheral blood lymphocytes that have been transduced with the retrovirus vector, *rkat4svgF3e-*, that encodes for a universal chimeric T cell receptor. (2) Determine the longevity of the genetically marked CD8(+) lymphocytes in the subject's peripheral blood. (Protocol #9403-069)

Appendix D-81. Dr. Joseph Rosenblatt of the University of California, Los Angeles, California, and Dr. Robert Seeger of Children's Hospital, Los Angeles, California, may conduct gene transfer experiments on 18 subjects with neuroblastoma (≤ 21 years of age). Patients at high risk of relapse with minimal or no detectable disease following myeloablative therapy and autologous bone marrow transplantation, or patients with progressive or persistent disease despite conventional therapy will be serially immunized with autologous or allogeneic neuroblastoma cells transduced to express γ interferon. Cells will be transduced with the retroviral vector, pHuy-IFN, encoding the human gene for γ interferon and lethally irradiated prior to use as an immunogen. The objectives of the study are: (1) to determine the maximum tolerable dose of transduced cells; (2) to determine the local, regional, and systemic toxicities of injected cells; and (3) to determine the antitumor response *in vivo* as measured by standard clinical tests and immunocytologic evaluation of marrow metastases. (Protocol #9403-068)

Appendix D-82. Dr. Kenneth L. Brigham of Vanderbilt University, Nashville, Tennessee, may conduct gene transfer experiments on 10 subjects (≤ 21 years of age) in two different patient protocols (5 for each protocol). Both protocols will use the same DNA/liposome preparations to deliver a plasmid DNA construct, pCMV4-AAT, encoding human alpha-1 antitrypsin gene driven by a cytomegalovirus promoter. In patients scheduled for elective pulmonary resection, the DNA/liposome complexes will be instilled through a fiber optic bronchoscope into a subsegment of the lung. Tissues of the lung will be obtained at the time of surgery. Transgene expression will be assessed by immunohistochemistry, *in situ* hybridization, and Western and Northern blot analyses. The effect of DNA/liposome complex administration on the histological appearance of the lung will also be evaluated. In patients with alpha-1 antitrypsin deficiency, the DNA/liposome complexes will be instilled into the nostril. Transgene expression will be determined in cells obtained by nasal lavage and nasal scraping, and the time course of transgene expression will be measured. The secretion of the alpha-1 antitrypsin protein in nasal fluid will be determined. Histological appearance of nasal mucosa will also be examined. The study will assess safety and feasibility of gene delivery to the human respiratory tract. (Protocol #9403-070)

Appendix D-83. Dr. H. Kim Lyerly of Duke University Medical Center, Durham, North Carolina, may conduct gene transfer experiments on 20 subjects with refractory or recurrent metastatic breast cancer (≥ 18 years of age). Autologous breast cancer cells will be transduced with the DNA/liposome complex, pMP6-IL2, containing a plasmid DNA vector derived from adeno-associated virus (AAV) that expresses the gene for human interleukin-2. Subjects will receive 4 subcutaneous injections of lethally irradiated tumor cells transduced with the DNA/liposome complex prior to injection. The objective of this study is to: (1) evaluate the safety and toxicity of the treatment, (2) determine the immunological effects, (3) determine the duration of clinical responses, and (4) measure patient survival. (Protocol #9409-086)

Appendix D-84. Drs. Flossie Wong-Staal, Eric Poeschla, and David Looney of the University of California at San Diego, La Jolla, California, may conduct gene transfer experiments on 6 subjects (≥ 18 and ≤ 65 years of age) infected with human immunodeficiency virus-1 (HIV-1). Autologous CD4(+) T lymphocytes will be transduced *ex vivo* with the retroviral vector, pMJT, expressing a hairpin ribozyme that cleaves the HIV-1 RNA in the 5' leader sequence. The transduced cells will be expanded and reinfused into the patients. The objectives of the study are: (1) to evaluate safety of reinfusing the transduced lymphocytes, (2) to compare (*in vivo*) the kinetics and survival of ribozyme-transduced cells with that of cells transduced with a control vector, (3) to determine *in vivo* expression of the ribozyme sequences in transduced lymphocytes, (4) to determine whether host immune responses directed against the transduced cells will occur *in vivo*, and (5) to obtain preliminary data on the effects of ribozyme gene therapy on *in vivo* HIV mRNA expression, viral burden and CD4(+) lymphocyte levels. (Protocol #9309-057)

Appendix D-85. Dr. Friedrich Schuening of the Fred Hutchinson Cancer Research Center, Seattle, Washington, may conduct gene transfer experiments on 10 subjects (≥ 18 years of age) with Type I Gaucher's disease. The peripheral blood repopulating cells (mobilized by patient pretreatment with recombinant granulocyte colony-stimulating factor) will be harvested and CD34(+) cells selected. CD34(+) cells will be transduced *ex vivo* with the retroviral vector, LgGC, that encodes human glucocerebrosidase cDNA. Following transduction, the transduced cells will be infused into the patient without myeloablative treatment. The primary endpoint of this study is to examine the safety of infusing CD34(+) cells transduced with the human glucocerebrosidase cDNA. Patients will be monitored for persistence and expression of the glucocerebrosidase gene in hematopoietic cells. (Protocol #9312-061)

Appendix D-86. Dr. Terence R. Flotte of the Johns Hopkins Children's Center, Baltimore, Maryland, may conduct gene transfer experiments on 16 subjects (≥ 18 years of age) with mild cystic fibrosis (CF). An adeno-associated virus (AAV) derived vector, encoding cystic fibrosis transmembrane conductance regulator (CFTR) gene, (tgAAVCF), will be administered to nasal (direct) and airway (bronchoscope) epithelial cells. This is a dose escalation study involving 8 cohorts. Each subject will receive both intranasal and bronchial administration of the adenoviral vector at 4 escalating doses. Nasal doses will range between 1×10^6 and 1×10^9 pfu. Lung administration will range between 1×10^7 and 1×10^{10} pfu. The primary goal of the study is to assess the safety of vector administration. Respiratory and nasal epithelial cells will be evaluated for gene transfer, expression, and physiologic correction. (Protocol #9409-083)

Appendix D-87. Drs. Jeffrey M. Isner and Kenneth Walsh of St. Elizabeth's Medical Center, Tufts University, Boston, Massachusetts, may conduct gene transfer experiments on 12 subjects (≥ 40 years of age) with peripheral artery disease (PAD). A plasmid DNA vector, pVEGF165, encoding the human gene for vascular endothelial growth factor (VEGF) will be used to express VEGF to induce collateral neovascularization. Percutaneous arterial gene transfer will be achieved using an angioplasty catheter with a hydrogel coated balloon to deliver the plasmid DNA vector to the artery. The objectives of the study are: (1) to determine the efficacy of arterial gene therapy to relieve rest pain and/or heal ischemic ulcers of the lower extremities in patients with PAD; and (2) to document the safety of the pVEGF arterial gene therapy for therapeutic angiogenesis. Subjects will undergo anatomic and physiologic examination to determine the extent of collateral artery development following pVEGF arterial gene therapy. (Protocol #9409-088)

Appendix D-88A. Dr. Ronald G. Crystal of New York Hospital-Cornell Medical Center, New York, New York, may conduct gene transfer experiments on 26 patients (≥ 15 years of age) with cystic fibrosis (CF). A replication deficient recombinant adenovirus vector will be used to transduce epithelial cells of the large bronchi with the E1/E3 deleted type 5 adenovirus vector, Ad_{GV}CFTR.10, which encodes the human cystic fibrosis transmembrane conductance regulator (CFTR) gene. The objective of this study is to define the safety and pharmacodynamics of CFTR gene expression in airway epithelial cells following single administration of escalating doses to the vector. If single administration is determined to be safe, subjects will undergo repeat administration to localized areas of the bronchi. (Protocol #9409-085)

Appendix D-88B. Drs. Eric J. Sorscher and James L. Logan of the University of Alabama, Birmingham, Alabama, may conduct gene transfer experiments on 9 subjects (≥ 18 years of age) with cystic fibrosis (CF). The normal human cystic fibrosis transmembrane conductance regulator (CFTR) gene will be expressed by a plasmid DNA vector, pKCTR, driven by the simian virus-40 (SV40) early gene promoter. The CFTR DNA construct will be delivered by cationic liposome-based gene transfer to nasal epithelial cells. The objectives of the study are to: (1) evaluate the safety of lipid-mediated gene transfer to nasal epithelial cells (including local inflammation and mucosal tissue); and (2) evaluate efficacy as determined by correction of the chloride ion transport defect, and wild-type CFTR mRNA and protein expression. (Protocol #9312-066)

Appendix D-89. Dr. Steven M. Albelda of the University of Pennsylvania Medical Center, Philadelphia, Pennsylvania, may conduct gene transfer experiments on 12 subjects with advanced mesothelioma. The adenovirus vector encoding the *Herpes simplex* virus thymidine kinase (HSV-TK) gene, H5.020RSVTK, will be administered through a chest tube to the pleural cavity. Tumor biopsies will be assayed for gene transfer and expression. Subjects will be monitored for immunological responses to the adenovirus vector. Ganciclovir will be administered intravenously 14 days following vector administration. The primary objective of this Phase I study is to evaluate the safety of direct adenovirus vector gene delivery to the pleural cavity of patients with malignant melanoma. (Protocol #9409-090)

Appendix D-90. Drs. Jeffrey Holt and Carlos B. Arteaga of the Vanderbilt University, Nashville, Tennessee, may conduct gene transfer experiments on 10 female patients (over 18 years of age) with metastatic breast cancer. Patient effusions from pleura or peritoneum will be drained and the fluid will be replaced with supernatant containing the retroviral vectors, XM6:antimyc or XM6:antifos, which express *c-myc* and *c-fos* antisense sequences, respectively, under the control of a mouse mammary tumor virus promoter. The objectives of this study are to: (1) assess uptake and expression of the vector sequences in breast cancer cells present in pleural and peritoneal fluids, and determine if this expression is tumor specific, (2) assess the safety of localized administration of antisense retroviruses, and (3) monitor subjects for clinical evidence of antitumor response. (Protocol #9409-084)

Appendix D-91. Dr. Jack A. Roth of MD Anderson Cancer Center, Houston, Texas, may conduct gene transfer experiments on 14 non-small cell lung cancer subjects (≥ 18 and ≤ 80 years of age) who have failed conventional therapy and who have bronchial obstruction. LNSX-based retroviral vectors containing the β -actin promoter will be used to express: (1) the antisense RNA of the *K-ras* oncogene (LN-K-*rasB*), and (2) the wildtype *p53* tumor suppressor gene (LNp53B). Tumor biopsies will be obtained to characterize *K-ras* and *p53* mutations. Relative to their specific mutation, subjects will undergo partial endoscopic resection of the tumor bed followed by bronchoscopic administration of the appropriate retrovirus construct. The objective of this study is to evaluate the safety and efficacy of intralesional administration of LN-K-*rasB* and LNp53 retrovirus constructs. (Protocol #9403-031)

Appendix D-92. Drs. Robert E. Sobol and Ivor Royston of the San Diego Regional Cancer Center, San Diego, California, may conduct gene transfer experiments on 12 subjects (≥ 18 years of age) with metastatic colon carcinoma. The autologous skin fibroblasts will be transduced with the retroviral vector, LNCX/IL-2, which encodes the gene for human interleukin-2 (IL-2). In this dose-escalation study, subjects will receive subcutaneous injections of lethally irradiated autologous tumor cells. The objectives of the study are to: (1) evaluate the safety of subcutaneous administration of LNCX/IL-2 transduced fibroblasts, (2) determine *in vivo* antitumor activity, and (3) monitor cellular and humoral antitumor responses. (Protocol #9312-060)

Appendix D-93. Dr. Michael Lotze of the University of Pittsburgh, Pittsburgh, Pennsylvania, may conduct gene transfer experiments on 18 subjects (≥ 18 years of age) with advanced melanoma, 6 with T-cell lymphoma, breast cancer, or head and neck cancer. Subjects should have accessible cutaneous tumors, and have failed standard therapy. Over 4 weeks, subjects will receive a total of 4 intratumoral injections of autologous fibroblasts transduced with the retrovirus vector, TFG-hIL-12-Neo. This vector, which consists of the murine MFG backbone, expresses both the p35 and p40 subunits of interleukin-12 (IL-12) and the *neo*^R selection marker. The objectives of the study are to: (1) define the local and systemic toxicity associated with peritumoral injections of gene-modified fibroblasts, (2) examine the local and systemic immunomodulatory effects of these injections, and (3) evaluate clinical antitumor efficacy. (Protocol #9406-081)

Appendix D-94. Drs. Evan Hersh, Emmanuel Akporiaye, David Harris, Alison Stopeck, Evan Unger, James Warneke, of the Arizona Cancer Center, Tucson, Arizona, may conduct gene transfer experiments on 25 subjects (≥ 18 years of age) with solid malignant tumors or lymphomas. A plasmid DNA/lipid complex designated as VCL-1102 (IL-2 Plasmid DNA/DMRIE/DOPE) will be used to transduce the human gene for interleukin-2 (IL-2). Patients with advanced cancer who have failed conventional therapy will undergo a procedure in which VCL-1102 is injected directly into the tumor mass to induce tumor-specific immunity. The objectives of the study are to: (1) determine safety and toxicity associated with escalating doses of VCL-1102; (2) confirm IL-2 expression in target cells; (3) determine biological activity and pharmacokinetics; and (4) determine whether IL-2 expression stimulates tumor regression in subjects with metastatic malignancies. (Protocol #9412-095)

Appendix D-95. Drs. Richard Morgan and Robert Walker of the National Institutes of Health, Bethesda, Maryland, may conduct gene transfer experiments on 48 human immunodeficiency virus (HIV) seropositive subjects (≥ 18 years of age). This Phase I/II study involves identical twins (one HIV seropositive and the other HIV seronegative). CD4(+) T cells will be enriched following apheresis of the HIV seronegative twin, induced to polyclonal proliferation with anti-CD3 and recombinant IL-2, transduced with either the LNL6/Neo^R or G1Na/Neo^R, and transduced with up to 2 additional retroviral vectors (G1RevTdSN and/or GCRTdSN(TAR)) containing potentially therapeutic genes (antisense TAR and/or transdominant Rev). These T cell populations will be expanded 10 to 1,000 fold in culture for 1 to 2 weeks and reinfused into the HIV seropositive twin. Subjects will receive up to 4 cycles of treatment using identical or different combinations of control and anti-HIV retrovirus vectors. The relative survival of these transduced T cell populations will be monitored by vector-specific polymerase chain reaction, while the subjects' functional immune status is monitored by standard *in vitro* and *in vivo* assays. (Protocol #9503-103)

Appendix D-96. Dr. Harry L. Malech of the National Institutes of Health, Bethesda, Maryland, may conduct gene transfer experiments on 2 subjects ≥ 18 years of age (with or without concurrent serious infection), and 3 subjects ≥ 18 years of age (with or without concurrent serious infection) or minors 13-17 years of age who have concurrent serious infection who have chronic granulomatous disease (CGD). CGD is an inherited immune deficiency disorder in which blood neutrophils and monocytes fail to produce antimicrobial oxidants (p47^{phox} mutation) resulting in recurrent life-threatening infections. Subjects will undergo CD34(+) mobilization with granulocyte colony stimulating factor (G-CSF). These CD34(+) cells will be transduced with the retrovirus vector, MFG-S-p47^{phox}, which encodes the gene for normal p47^{phox}. The objectives of this study are to: (1) determine the safety of administering MFG-S-p47^{phox} transduced CD34(+) cells, and (2) demonstrate increased functional oxidase activity in circulating neutrophils. (Protocol #9503-104)

Appendix D-97. Drs. Chris Evans and Paul Robbins of the University of Pittsburgh, Pittsburgh, Pennsylvania, may conduct gene transfer experiments on 6 subjects (≥ 18 and ≤ 76 years of age) with rheumatoid arthritis. Rheumatoid arthritis is a chronic, progressive disease thought to be of autoimmune origin. A gene encoding an interleukin-1 receptor antagonist protein (IRAP) will be delivered to the rheumatoid metacarpal-phalangeal joints to determine the autoimmune reactions can be interrupted. The vector construct, DFG-IRAP, is based on the MFG murine retrovirus vector backbone, and encodes the human IRAP gene. Synovial fibroblasts will be generated from the rheumatoid arthritic joint tissue obtained from patients who are scheduled to undergo surgery. The fibroblasts will be transduced with the DFG-IRAP vector, and the transduced cells injected into the synovial space. The synovial fluid and joint material will be collected 7 days later to determine the presence and location of the transduced synovial fibroblasts and the level of IRAP in the joint fluid. (Protocol 9406-074)

Appendix D-98. Dr. R. Scott McIvor of the University of Minnesota, Minneapolis, Minnesota, may conduct gene transfer experiments on 2 children with purine nucleoside phosphorylase (PNP) deficiency. PNP deficiency results in severe T-cell immunodeficiency, an autosomal recessive inherited disease which is usually fatal in the first decade of life. Autologous peripheral blood lymphocytes will be cultured in an artificial capillary cartridge in the presence of anti-CD3 monoclonal antibody and interleukin-2 and transduced with the retroviral vector, LPNSN-2, encoding human PNP. Subjects will undergo bimonthly intravenous administration of transduced T cells for a maximum of 1 year. The objectives of the study are to determine: (1) the safety of intravenous administration of transduced T cells in children with PNP deficiency, (2) the efficiency of PNP gene transfer and duration of gene expression *in vivo*, and (3) the effect of PNP gene transfer on immune function. (Protocol #9506-110)

Appendix D-99. Drs. Nikhil C. Munshi and Bart Barlogie of the University of Arkansas School for Medical Sciences, Little Rock, Arkansas, may conduct gene transfer experiments on 21 subjects (>18 and <65 years of age) with relapsed or persistent multiple myeloma who are undergoing T cell depleted allogeneic bone marrow transplantation. Donor peripheral blood lymphocytes will be cultured in vitro with interleukin-2 and anti-CD3 monoclonal antibody. T cell depleted lymphocytes will be transduced with the retroviral construct, G1Tk1SvNa.7, which encodes the Herpes simplex virus thymidine kinase (HSV-TK) gene. The transduced cells will be reinfused. In this dose escalation study, 3 subjects will undergo cell-mediated gene transfer per cohort (maximum of 5 cohorts) until Grade III or IV Graft versus Host Disease (GVHD) is observed. A maximum of 6 additional patients may be entered at that maximum tolerated dose. The objectives of this study are to determine the: (1) safety of transduced donor cell infusions, (2) effectiveness of donor cell infusions in decreasing the effects of severe GVHD, (3) effectiveness of donor cell infusions in prolonging multiple myeloma remission, and (4) effectiveness of ganciclovir in eliminating donor cells for the purpose of preventing the depletion of erythrocytes. (Protocol #9506-107)

Appendix D-100. Dr. Wayne A. Marasco of Dana-Farber Cancer Institute, Boston, Massachusetts, may conduct gene transfer experiments on 6 subjects (≥ 18 and ≤ 65 years of age) with human immunodeficiency virus type-1 (HIV-1). Autologous lymphocytes from asymptomatic subjects will be transduced *ex vivo* with a retroviral vector, LNCs105, encoding the sFv105 antibody specific for the HIV-1 envelope protein. An identical aliquot will be simultaneously transduced with a control retroviral vector lacking the sFv105 cassette. Transduced cells will be reinfused into patients and the differential survival of both populations of CD4+ lymphocytes compared. The objective of the study is to determine whether the intracellular expression of a human single chain antibody against HIV-1 envelope glycoprotein gp160 that blocks gp160 processing and the production of infectious virions can safely prolong the survival of CD4(+) lymphocytes in HIV-1-infected subjects. (Protocol #9506-111)

Appendix D-101. Dr. Henry Dorkin of the New England Medical Center, Boston, Massachusetts, and Dr. Allen Lapey of Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, propose to conduct gene transfer experiments on 16 subjects (≥ 18 years of age). An E1/partial E4-deleted, replication-deficient, type 2 adenovirus vector, AD2/CFTR-2, will be used to deliver the human cystic fibrosis transmembrane conductance regulator (CFTR) gene by aerosol administration (nebulization) to the lung of CF patients. Aerosol administration will be initiated only after initial safety data has been obtained from the lobar administration protocol (#9409-091). This is a single administration dose-escalation study in which subjects will receive between 8×10^6 and 2.5×10^{10} pfu. Subjects will be assessed for evidence of adverse, systemic, immune, inflammatory, or respiratory effects in response to AD2/CFTR-2. Subjects will be monitored for virus shedding and transgene expression. Health care workers present in the facility will be required to sign an Informed Consent document regarding the possibility of virus transmission. (Protocol #9412-074)

Appendix D-102. Drs. Charles J. Link and Donald Moorman of the Human Gene Therapy Research Institute, Des Moines, Iowa, may conduct gene transfer experiments on 24 female subjects (≥ 18 years of age) with refractory or recurrent ovarian cancer. Subjects will undergo intraperitoneal delivery (via Tenkhoff catheter) of the vector producing cells (VPC), PA317/LTKOSN.2. These VPC express the *Herpes simplex* virus thymidine kinase (HSV-TK) gene which confers sensitivity to killing by the antiviral drug, ganciclovir (GCV). The LTKOSN.2 retrovirus vector is based on the LXSN backbone. Two weeks following intraperitoneal delivery of the VPC, subjects will receive 5 mg/kg intravenous GCV twice daily for 14 days. Subjects will receive between 1×10^5 and 1×10^8 VPC/kg in this dose escalation study. Subjects will be evaluated by X-ray and peritoneoscopy of the abdomen for evidence of clinical response. The objectives of this study are to determine the safety of intraperitoneal VPC administration. (Protocol #9503-100)

Appendix D-103. Dr. David T. Curiel of the University of Alabama, Birmingham, Alabama, may conduct gene transfer experiment of 15 subjects (≥ 18 years of age) with metastatic colorectal cancer. Subjects will receive intramuscular injection of the polynucleotide vaccine, pGT63, which is a plasmid DNA vector expressing carcinoembryonic antigen (CEA) and hepatitis B surface antigen (HBsAg). The objectives of the study are to: (1) characterize the immune response to CEA and HBsAg following a single intramuscular injection and following 3 consecutive intramuscular injections, and (2) determine the safety of intramuscular injection of the plasmid DNA vector at doses ranging between 0.1 to 1.0 milligrams (single dose) and 0.9 to 3.0 milligrams (total multidose). (Protocol #9506-073)

Appendix D-104. Dr. Chester B. Whitley of the University of Minnesota, Minneapolis, Minnesota, may conduct gene transfer experiments on two adult subjects (18 years of age or older) with mild Hunter syndrome (Mucopolysaccharidosis Type II). The autologous peripheral blood lymphocytes will be transduced *ex vivo* with the retroviral vector, L2SN, encoding the human cDNA for iduronate-2-sulfatase (IDS). The transduced lymphocytes will be reinfused into the patients on a monthly basis. The study will determine the frequency of peripheral blood lymphocyte transduction and the half-life of the infused cells. Evaluation of patients will include measurement of blood levels of IDS enzyme, assessment of metabolic correction by urinary glycosaminoglycan levels, clinical response of the disease, and monitoring for potential toxicity. This Phase I study is to demonstrate the safety of the L2SN-mediated gene therapy and to provide a preliminary evaluation of clinical efficacy. (Protocol #9409-087)

Appendix D-105. Drs. James Economou, John Glaspy, and William McBride of the University of California, Los Angeles, California, may conduct gene transfer experiments on 25 subjects (≥ 18 years of age) with metastatic melanoma. The protocol is an open label, Phase I trial to evaluate the safety and immunological effects of administering lethally irradiated allogeneic and autologous melanoma cells transduced with the retroviral vector, IL-7/HyTK, which encodes the gene for human interleukin-7 (IL-7). Subjects will receive 1×10^7 irradiated unmodified autologous tumor cells in combination with escalating doses of IL-7/HyTK transduced allogeneic melanoma cells (M24 cell line). The number of M24 cells administered will be adjusted based on the level of IL-7 expression. Subjects will receive 3 biweekly subcutaneous injections of M24 cells expressing 10, 100, or 1000 nanograms of IL-7/hour *in vivo*. A final cohort of 5 subjects will receive IL-7/HyTK transduced autologous cells. Subjects will be monitored for antitumor activity by skin tests, biopsy analysis, tumor-specific antibody activity, and cytotoxic T lymphocyte precursor evaluation. Non-immunologic parameters will also be monitored. (Protocol #9503-101)

Appendix D-106. Dr. Jack A. Roth, MD Anderson Cancer Center, may conduct gene transfer experiments on 42 subjects (≥ 18 years of age) with refractory non-small cell lung cancer (NSCLC). Subjects will receive direct intratumoral injection of a replication-defective type 5 adenovirus vector, AD5CMV-p53, to deliver the normal human *p53* tumor suppressor gene. The E1 region of AD5CMV-p53 has been replaced with a *p53* expression cassette containing the human cytomegalovirus promoter (CMV). Subjects will be divided into 2 treatment groups: (1) 21 subjects will receive Ad5CMV-p53 alone, and (2) 21 subjects will receive Ad5CMV-p53 in combination with cisplatin. Following vector administration, subjects will be isolated for 96 hours during which time, assays will be conducted to demonstrate the lack of shedding of adenovirus vector. The objectives of this study are determine: (1) the maximum tolerated dose of AD5CMV-p53, (2) qualitative and quantitative toxicity related to vector administration, and (3) biologic activity.

Prior to administration, adenovirus vector stocks will be screened for *p53* mutants using the SAOS osteosarcoma cell assay that was submitted by Dr. Roth on June 23, 1995. This biologic assay compares the activity of a standard stock of Adp53 vector to the activity of newly produced stocks. The standard stock of Adp53 will be defined as mediating cell death in 100% of SAOS cells (human osteosarcoma cell line with homozygous *p53* deletion) at an MOI of 50:1 (titer $> 5 \times 10^{10}$) on day 5 of culture. The sensitivity of the assay for detecting inactive (presumed mutant) Adp53 vector will be determined by adding increasing amounts of Adluc (control adenovirus vector containing the luciferase gene) to the Adp53 stock to determine the percentage of inactive vector required to decrease growth inhibition of SAOS cells mediated by Adp53. The test lot of Adp53 will be tested for its ability to inhibit SAOS in a 5 day assay. Significant loss of inhibitory activity compared with the standard would indicate an unacceptable level of inactive (presumed mutant) vector. (Protocol #9406-079)

Appendix D-107A. Dr. Gary Clayman, M.D. Anderson Cancer Center, Houston, Texas, may conduct gene transfer experiments on 21 subjects (≥ 18 years of age) with refractory squamous cell carcinoma of the head and neck. Subjects will receive direct intratumoral injection of a replication-defective type 5 adenovirus vector, AD5CMV-p53, to deliver the normal human *p53* tumor suppressor gene. The E1 region of AD5CMV-p53 has been replaced with a *p53* expression cassette containing the human cytomegalovirus promoter (CMV). Subjects will be divided into 2 treatment groups: (1) those with non-resectable tumors, and (2) those with surgically accessible tumors. Subjects will receive multiple injections of vector in each dose-escalation cohort. Following vector administration, subjects will be isolated for 48 hours during which time, assays will be conducted to demonstrate the lack of shedding of adenovirus vector. The objectives of the study are to determine: (1) the maximum tolerated dose of AD5CMV-p53, (2) qualitative and quantitative toxicity related to vector administration, and (3) biologic activity.

Prior to administration, adenovirus vector stocks will be screened for p53 mutants using the SAOS osteosarcoma cell assay that was submitted by Dr. Roth on June 23, 1995. This biologic assay compares the activity of a standard stock of Adp53 vector to the activity of newly produced stocks. The standard stock of Adp53 will be defined as mediating cell death in 100% of SAOS cells (human osteosarcoma cell line with homozygous p53 deletion) at an MOI of 50:1 (titer $> 5 \times 10^{10}$) on day 5 of culture. The sensitivity of the assay for detecting inactive (presumed mutant) Adp53 vector will be determined by adding increasing amounts of Adluc to the Adp53 stock to determine the percentage of inactive vector required to decrease growth inhibition of SAOS cells mediated by Adp53. The test lot of Adp53 will be tested for its ability to inhibit SAOS in a 5 day assay. Significant loss of inhibitory activity compared with the standard would indicate an unacceptable level of inactive (presumed mutant) vector. (Protocol #9412-096)

Appendix D-107B. Drs. Bernard A. Fox and Walter J. Urba of Earle A. Chiles Research Institute, Providence Medical Center, Portland, Oregon, may conduct gene transfer experiments on 18 subjects (≥ 18 years of age) with metastatic renal cell carcinoma or melanoma. Autologous tumor cells will be surgically removed, transduced *in vitro* with the cationic liposome plasmid vector, VCL-1005, which encodes human leukocyte antigen (HLA)-B7 and beta-2 microglobulin. Subjects will receive subcutaneous injection of lethally irradiated transduced cells in one limb. The contralateral limb will be injected with lethally irradiated untransduced tumor cells in combination with Bacille Calmette-Guerin (BCG). Approximately 21 days following tumor cell injection, subjects will undergo lymphadenectomy for subsequent *in vitro* expansion of anti-CD3 activated lymphocytes. Activated lymphocytes will be adoptively transferred on approximately day 35 in combination with a 5-day course of interleukin-2 (IL-2). On approximately day 45, subjects will receive a second cycle of IL-2. The objectives of this study are to determine: (1) the safety of administering anti-CD3 activated antitumor effector T cells in draining lymph nodes, and (2) whether HLA-B7/ β -2 gene transfer augments the sensitization of anti-tumor effector T-cells in draining lymph nodes. (Protocol 9506-108)

Appendix D-108. Dr. Mitchell S. Steiner, University of Tennessee, Memphis, Tennessee, and Dr. Jeffrey T. Holt, Vanderbilt University School of Medicine, Nashville, Tennessee, may conduct gene transfer experiments on 15 male subjects (35 to 75 years of age) with metastatic prostate cancer. Malignant cells obtained from advanced prostate cancer subjects have been demonstrated to express high levels of the protooncogene c-myc *in vivo*. The mouse mammary tumor virus (MMTV) long terminal repeat (LTR) is expressed at high levels in prostate tissue. Following removal of malignant cells via biopsy, subjects will receive a single transrectal ultrasound-guided intraprostate quadrant injection of the retrovirus vector, XM6:MMTV-antisense c-myc, for 4 consecutive days at the site of the original biopsy. The objectives of this Phase I study are to: (1) quantitatively assess the uptake and expression of XM6:MMTV-antisense c-myc by prostate cancer cells *in vivo*, (2) determine whether c-myc gene expression is prostate tumor-specific, (3) assess safety of intraprostate injection of XM6:MMTV-antisense c-myc, and (4) biologic efficacy (antisense inhibition of tumor growth). (Protocol #9509-123)

Appendix D-109. Drs. Ronald G. Crystal, Edward Hershowitz, and Michael Lieberman, New York Hospital-Cornell Medical Center, New York, New York, may conduct gene transfer experiments on 18 subjects (18 to 70 years of age) with metastatic colon carcinoma with liver metastases. In this Phase I dose-escalation study, subjects will receive computed tomography (CT)-guided intratumoral injections of the adenovirus vector, Ad_{GV}CD.10, into the same hepatic metastasis in 4 equal volumes (100 microliters), each with a separate entry into the liver. This dosage schedule will be performed on Days 1 and 7. 5-fluorocytosine (200 milligrams/kilogram/24 hours) will be administered orally in 4 equal doses starting on day 2 and continuing through the time of laparotomy. The objectives of this study are to: (1) determine the dose-dependent toxicity of direct administration of Ad_{GV}CD.10 to hepatic metastases combined with oral administration of 5-fluorocytosine, (2) quantitatively assess transfer and expression of the cytosine deaminase gene in target cells, and (3) determine the biologic effects of direct Ad_{GV}CD.10 administration to hepatic metastases. (Protocol #9509-125)

Appendix D-110. Drs. Andres Berchuck and H. Kim Lyerly of Duke University Medical Center, Durham, North Carolina, may conduct gene transfer experiments on 18 subjects (≥ 18 years of age) with refractory metastatic ovarian cancer. Autologous tumor cells obtained from ascites or surgically removed tumor will be transduced with the cationic liposome vector, PMP6A-IL2, that contains an adeno-associated virus derived plasmid DNA, a cytomegalovirus (CMV) promoter, and interleukin-2 (IL-2) complementary DNA (cDNA). In this dose-escalation study, subjects will undergo 4 cycles of intradermal injections (thigh or abdomen) of *ex vivo* transduced, lethally irradiated tumor cells in an attempt to induce an antitumor response. The objectives of the study are to evaluate: (1) the safety of intradermally injected transduced cells, and (2) antitumor response following therapy. (Protocol #9506-110)

Appendix D-111. Drs. Stephen L. Eck and Jane B. Alavi of the University of Pennsylvania Medical Center, Philadelphia, Pennsylvania, may conduct gene transfer experiments on 18 subjects (>18 years of age) with malignant glioma. The adenovirus vector encoding the *Herpes simplex* virus thymidine kinase (HSV-TK) gene, H5.020RSVTK, will be injected by a stereotactic guided technique into brain tumors. Afterwards, the patients will receive systemic ganciclovir (GCV) treatment. Patients eligible to undergo a palliative debulking procedure will receive the same treatment followed by resection on day 7, and a second dose of the vector intra-operatively. Brain tissues removed by resection will be analyzed for adenovirus infection, transgene expression, and signs of inflammation. The size and metabolic activity of tumors will be monitored by scanning with magnetic resonance imaging and positron emission tomography. The objective of the study is to evaluate the overall safety of this treatment and to gain insight into the parameters that may limit the general applicability of this approach. (Protocol #9409-089)

Appendix D-112. Drs. Robert Grossman and Savio Woo of the Baylor College of Medicine & Methodist Hospital, Houston, Texas, may conduct gene transfer experiments on 20 subjects (≥ 18 years of age) with refractive central nervous system malignancies. Subjects will receive stereotaxic injections of a replication-defective, type 5 E1/E3-deleted adenovirus vector, ADV/RSV-tk, to deliver the *Herpes simplex* virus thymidine kinase (HSV-TK) gene to tumor cells. Expression of the HSV-TK gene is driven by a Rous sarcoma virus long terminal repeat (RSV-LTR). Subjects will receive a single time-course of intravenous ganciclovir (GCV) (14 consecutive days) following vector administration. Following demonstration of safety with the initial starting dose of 1×10^8 particles in 5 subjects, additional cohorts will receive between 5×10^8 and 1.5×10^9 particles. Each cohort will be monitored for toxicity for one month before administration of the next higher dose to subsequent cohorts. Subjects will be monitored for evidence of clinical efficacy by magnetic resonance imaging and/or computer tomography scans. The primary objective of this Phase I study is to determine the safety of vector administration. (Protocol #9412-098)

Appendix D-113. Drs. Gabriel N. Hortobagyi, Gabriel Lopez-Berestein, and Mien-Chie Hung, of the University of Texas MD Anderson Cancer Center, Houston, Texas, may conduct gene transfer experiments on a maximum of 24 adult patients (12 for each cancer) with metastatic breast or ovarian carcinoma. Overexpression of the *HER-2/neu* oncogene occurs in 30% of ovarian and breast cancers, and it is associated with enhanced metastatic potential, drug resistance, and poor survival. The E1A gene of the adenovirus type 5 functions as a tumor suppressor gene when transfected into cancer cells which overexpress the *HER-2/neu* oncogene. E1A expression induces down regulation of the level of the *HER-2/neu* oncoprotein by a transcriptional control mechanism. A plasmid, pE1A, encoding the adenovirus E1A gene with its own promoter will be administered as a DNA/lipid complex via the intraperitoneal or intrapleural route. The objectives of the study are: (1) to determine E1A gene transduction into malignant cells after the administration of E1A/lipid complex by intrapleural or intraperitoneal administration, (2) to determine whether E1A gene therapy can down-regulate *HER-2/neu* expression after intrapleural or intraperitoneal administration, (3) to determine the maximum biologically active dose (MBAD), or the maximum tolerated dose (MTD) of E1A/lipid complex, (4) to determine the toxicity and tolerance of E1A/lipid complex administered into the pleural or peritoneal space, and to assess the reversibility of such toxicity, and (5) to evaluate tumor response. (Protocol #9512-137)

Appendix D-114. Drs. Keith L. Black and Habib Fakhrai of the University of California, Los Angeles, California, may conduct gene transfer experiments on 12 subjects (≥ 18 years of age) with glioblastoma multiforme. An Epstein-Barr virus (EBV) based plasmid vector, pCEP-4/TGF- β 2 antisense, encoding antisense RNA will be used to inhibit TGF- β 2 production. Tumor samples obtained from the patients at the time of clinically indicated surgery will be grown in culture to establish a cell line for each patient. The patients' tumor cells will be genetically altered with the pCEP-4/TGF- β 2 vector to inhibit their secretion of TGF- β . Following completion of the traditional post surgical radiation therapy, the first cohort of patients will receive, at 3 week intervals, 4 injections of 5×10^6 irradiated gene modified autologous tumor cells. Subsequently, in dose escalation studies, the second cohort will receive 1×10^7 cells, and the third cohort, 2×10^7 cells. The results of this Phase I trial will be used to assess the safety of this form of gene therapy and may provide preliminary data to evaluate the potential utility of TGF- β 2 antisense gene therapy in the management of gliomas. (Protocol #9512-138)

Appendix D-115. Dr. Ronald G. Crystal of New York Hospital-Cornell Medical Center, New York, New York, may conduct gene transfer experiments on a total of 21 (with an option for an additional 5) normal males and female subjects, age ≥ 18 years. Replication-deficient adenovirus (Ad) vector previously has been used in a number of human gene therapy strategies to transfer genes *in vivo* for therapeutic purposes. The purpose of this protocol is to characterize the local (skin), systemic (blood), and distant compartment (lung) immunity in normal individuals after intradermal administration of a replication deficient Ad5-based vector, named

Ad_{GV}CD.10, carrying the gene coding for the *E. coli* enzyme, cytosine deaminase (CD). Following intradermal administration of the vector to normal individuals, the skin, blood, and lung immune responses to the Ad vector and CD transgene will be evaluated over time. This vector has been safely administered intrahepatically ten times to five individuals with colon carcinoma. No adverse effects in Protocol #9509-125 have been observed. The present protocol will yield insights into normal human immune responses to both the Ad vector, as well as to a heterologous (i.e., non-human) gene product (CD). Note: This study is designed to answer basic biological questions regarding characterization of the immune responses to such vectors that have been previously documented. (Protocol #9701-171)

Appendix D-116. Dr. Daniel Rockey at Oregon State University and Dr. Walter Stamm at the University of Washington may conduct experiments to deliberately transfer a gene encoding tetracycline resistance from *Chlamydia suis* (a swine pathogen) into *C. trachomatis* (a human pathogen). This approval is specific to Drs. Rockey and Stamm and research with these resistant organisms may only occur under the conditions as specified by the NIH Director (72 FR 61661). This approval was effective as of September 24, 2007.

Appendix D-117. Dr. David Walker at the University of Texas Medical Branch may conduct experiments to deliberately introduce a gene encoding chloramphenicol resistance into *Rickettsia conorii*. This approval is specific to Dr. Walker and research with these resistant organisms may only occur under the conditions as specified by the NIH Director (73 FR 32719). This approval was effective as of April 7, 2008.

Appendix D-118. Dr. Harlan Caldwell at the Rocky Mountain Laboratories may conduct experiments to deliberately introduce a gene encoding tetracycline resistance into *Chlamydia trachomatis* serovar L2. This approval is specific to Dr. Caldwell and research with this resistant organism may only occur under the conditions as specified by the NIH Director (76 FR 27653). This approval was effective as of April 26, 2010.

APPENDIX E. CERTIFIED HOST-VECTOR SYSTEMS (See Appendix I, Biological Containment)

While many experiments using *Escherichia coli* K-12, *Saccharomyces cerevisiae*, and *Bacillus subtilis* are currently exempt from the NIH Guidelines under Section III-F, Exempt Experiments, some derivatives of these host-vector systems were previously classified as Host-Vector 1 Systems or Host-Vector 2 Systems. A listing of those systems follows:

Appendix E-I. *Bacillus subtilis*

Appendix E-I-A. *Bacillus subtilis* Host-Vector 1 Systems

The following plasmids are accepted as the vector components of certified *B. subtilis* systems: pUB110, pC194, pS194, pSA2100, pE194, pT127, pUB112, pC221, pC223, and pAB124. *B. subtilis* strains RUB 331 and BGSC 1S53 have been certified as the host component of Host-Vector 1 systems based on these plasmids.

Appendix E-I-B. *Bacillus subtilis* Host-Vector 2 Systems

The asporogenic mutant derivative of *Bacillus subtilis*, ASB 298, with the following plasmids as the vector component: pUB110, pC194, pS194, pSA2100, pE194, pT127, pUB112, pC221, pC223, and pAB124.

Appendix E-II. *Saccharomyces cerevisiae*

Appendix E-II-A. *Saccharomyces cerevisiae* Host-Vector 2 Systems

The following sterile strains of *Saccharomyces cerevisiae*, all of which have the ste-VC9 mutation, SHY1, SHY2, SHY3, and SHY4. The following plasmids are certified for use: Ylp1, YEp2, YEp4, Ylp5, YEp6, YRp7, YEp20, YEp21, YEp24, Ylp25, Ylp26, Ylp27, Ylp28, Ylp29, Ylp30, Ylp31, Ylp32, and Ylp33.

Appendix E-III. *Escherichia coli***Appendix E-III-A. *Escherichia coli* (EK2) Plasmid Systems**

The *Escherichia coli* K-12 strain chi-1776. The following plasmids are certified for use: pSC101, pMB9, pBR313, pBR322, pDH24, pBR325, pBR327, pGL101, and pHB1. The following *Escherichia coli*/S. cerevisiae hybrid plasmids are certified as EK2 vectors when used in *Escherichia coli* chi-1776 or in the sterile yeast strains, SHY1, SHY2, SHY3, and SHY4: YIp1, YEp2, YEp4, YIp5, YEp6, YRp7, YEp20, YEp21, YEP24, YIp25, YIp26, YIp27, YIp28, YIp29, YIp30, YIp31, YIp32, and YIp33.

Appendix E-III-B. *Escherichia coli* (EK2) Bacteriophage Systems

The following are certified EK2 systems based on bacteriophage lambda:

Vector	Host
λgt WESλB'	DP50 ^{supF}
λgt WESλB*	DP50 ^{supF}
λgt ZJ virλB'	<i>Escherichia coli</i> K-12
λgtALO·λB	DP50 ^{supF}
Charon 3A	DP50 or DP50 ^{supF}
Charon 4A	DP50 or DP50 ^{supF}
Charon 16A	DP50 or DP50 ^{supF}
Charon 21A	DP50 ^{supF}
Charon 23A	DP50 or DP50 ^{supF}
Charon 24A	DP50 or DP50 ^{supF}

Escherichia coli K-12 strains chi-2447 and chi-2281 are certified for use with lambda vectors that are certified for use with strain DP50 or DP50^{supF} provided that the *su*-strain not be used as a propagation host.

Appendix E-IV. *Neurospora crassa***Appendix E-IV-A. *Neurospora crassa* Host-Vector 1 Systems**

The following specified strains of *Neurospora crassa* which have been modified to prevent aerial dispersion:

In1 (inositol-less) strains 37102, 37401, 46316, 64001, and 89601. Csp-1 strain UCLA37 and csp-2 strains FS 590, UCLA101 (these are conidial separation mutants).

Eas strain UCLA191 (an "easily wettable" mutant).

Appendix E-V. *Streptomyces***Appendix E-V-A. *Streptomyces* Host-Vector 1 Systems**

The following *Streptomyces* species: *Streptomyces coelicolor*, *S. lividans*, *S. parvulus*, and *S. griseus*. The following are accepted as vector components of certified *Streptomyces* Host-Vector 1 systems: *Streptomyces* plasmids SCP2, SLP1.2, pIJ101, actinophage phi C31, and their derivatives.

Appendix E-VI. *Pseudomonas putida***Appendix E-VI-A. *Pseudomonas putida* Host-Vector 1 Systems**

Pseudomonas putida strains KT2440 with plasmid vectors pKT262, pKT263, and pKT264.

APPENDIX F. CONTAINMENT CONDITIONS FOR CLONING OF GENES CODING FOR THE BIOSYNTHESIS OF MOLECULES TOXIC FOR VERTEBRATES

Appendix F-I. General Information

Appendix F specifies the containment to be used for the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates. The cloning of genes coding for molecules toxic for vertebrates that have an LD₅₀ of < 100 nanograms per kilograms body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, *Shigella dysenteriae* neurotoxin) are covered under [Section III-B-1 \(Experiments Involving the Cloning of Toxin Molecules with LD₅₀ of Less than 100 Nanograms Per Kilogram Body Weight\)](#) and require Institutional Biosafety Committee and NIH/OBA approval before initiation. No specific restrictions shall apply to the cloning of genes if the protein specified by the gene has an LD₅₀ ≥ 100 micrograms per kilograms of body weight. Experiments involving genes coding for toxin molecules with an LD₅₀ of < 100 micrograms per kilograms and > 100 nanograms per kilograms body weight require Institutional Biosafety Committee approval and registration with NIH/OBA prior to initiating the experiments. A list of toxin molecules classified as to LD₅₀ is available from NIH/OBA. Testing procedures for determining toxicity of toxin molecules not on the list are available from the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). The results of such tests shall be forwarded to NIH/OBA, which will consult with *ad hoc* experts, prior to inclusion of the molecules on the list (see [Section IV-C-1-b-\(2\)-\(c\), Minor Actions](#)).

Appendix F-II. Cloning of Toxin Molecule Genes in *Escherichia coli* K-12

Appendix F-II-A. Cloning of genes coding for molecules toxic for vertebrates that have an LD₅₀ of >100 nanograms per kilograms and <1000 nanograms per kilograms body weight (e.g., abrin, *Clostridium perfringens* epsilon toxin) may proceed under Biosafety Level (BL) 2 + EK2 or BL3 + EK1 containment conditions.

Appendix F-II-B. Cloning of genes for the biosynthesis of molecules toxic for vertebrates that have an LD₅₀ of >1 microgram per kilogram and <100 microgram per kilogram body weight may proceed under BL1 + EK1 containment conditions (e.g., *Staphylococcus aureus* alpha toxin, *Staphylococcus aureus* beta toxin, ricin, *Pseudomonas aeruginosa* exotoxin A, *Bordetella pertussis* toxin, the lethal factor of *Bacillus anthracis*, the *Pasteurella pestis* murine toxins, the oxygen-labile hemolysins such as streptolysin O, and certain neurotoxins present in snake venoms and other venoms).

Appendix F-II-C. Some enterotoxins are substantially more toxic when administered enterally than parenterally. The following enterotoxins shall be subject to BL1 + EK1 containment conditions: cholera toxin, the heat labile toxins of *Escherichia coli*, *Klebsiella*, and other related proteins that may be identified by neutralization with an antiserum monospecific for cholera toxin, and the heat stable toxins of *Escherichia coli* and of *Yersinia enterocolitica*.

Appendix F-III. Cloning of Toxic Molecule Genes in Organisms Other Than *Escherichia coli* K-12

Requests involving the cloning of genes coding for toxin molecules for vertebrates at an LD₅₀ of <100 nanograms per kilogram body weight in host-vector systems other than *Escherichia coli* K-12 will be evaluated by NIH/OBA in consultation with *ad hoc* toxin experts (see [Sections III-B-1, Experiments Involving the Cloning of Toxin Molecules with LD₅₀ of Less than 100 Nanograms Per Kilogram Body Weight](#), and [IV-C-1-b-\(2\)-\(c\), Minor Actions](#)).

Appendix F-IV. Specific Approvals

An updated list of experiments involving the deliberate formation of recombinant DNA containing genes coding for toxins lethal for vertebrates at an LD₅₀ of <100 nanograms per kilogram body weight is available from the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

APPENDIX G. PHYSICAL CONTAINMENT

Appendix G specifies physical containment for standard laboratory experiments and defines Biosafety Level 1 through Biosafety Level 4. For large-scale (over 10 liters) research or production, Appendix K (*Physical Containment for Large Scale Uses of Organisms Containing Recombinant DNA Molecules*) supersedes Appendix G. [Appendix K](#) defines Good Large Scale Practice through Biosafety Level 3 - Large Scale. For certain work with plants, Appendix P (*Physical and Biological Containment for Recombinant DNA Research Involving Plants*) supersedes Appendix G. [Appendix P](#) defines Biosafety Levels 1 through 4 - Plants. For certain work with animals, Appendix Q (*Physical and Biological Containment for Recombinant DNA Research Involving Animals*) supersedes Appendix G. [Appendix Q](#) defines Biosafety Levels 1 through 4 - Animals.

Appendix G-I. Standard Practices and Training

The first principle of containment is strict adherence to good microbiological practices (see [Appendices G-III-A](#) through [G-III-J](#), *Footnotes and References of Appendix G*). Consequently, all personnel directly or indirectly involved in experiments using recombinant DNA shall receive adequate instruction (see [Sections IV-B-1-h](#), *Responsibilities of the Institution--General Information*, and [IV-B-7-d](#), *Responsibilities of the Principal Investigator Prior to Initiating Research*). At a minimum, these instructions include training in aseptic techniques and in the biology of the organisms used in the experiments so that the potential biohazards can be understood and appreciated.

Any research group working with agents that are known or potential biohazards shall have an emergency plan that describes the procedures to be followed if an accident contaminates personnel or the environment. The Principal Investigator shall ensure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan (see [Sections IV-B-7-d](#), *Responsibilities of the Principal Investigator Prior to Initiating Research* and [IV-B-7-e](#), *Responsibilities of the Principal Investigator During the Conduct of the Research*). If a research group is working with a known pathogen for which there is an effective vaccine, the vaccine should be made available to all workers. Serological monitoring, when clearly appropriate, will be provided (see [Section IV-B-1-f](#), *Responsibilities of the Institution--General Information*).

The *Laboratory Safety Monograph* (see [Appendix G-III-O](#), *Footnotes and References of Appendix G*) and *Biosafety in Microbiological and Biomedical Laboratories* (see [Appendix G-III-A](#), *Footnotes and References of Appendix G*) describe practices, equipment, and facilities in detail.

Appendix G-II. Physical Containment Levels

The objective of physical containment is to confine organisms containing recombinant DNA molecules and to reduce the potential for exposure of the laboratory worker, persons outside of the laboratory, and the environment to organisms containing recombinant DNA molecules. Physical containment is achieved through the use of laboratory practices, containment equipment, and special laboratory design. Emphasis is placed on primary means of physical containment which are provided by laboratory practices and containment equipment. Special laboratory design provides a secondary means of protection against the accidental release of organisms outside the laboratory or to the environment. Special laboratory design is used primarily in facilities in which experiments of moderate to high potential hazard are performed.

Combinations of laboratory practices, containment equipment, and special laboratory design can be made to achieve different levels of physical containment. Four levels of physical containment, which are designated as BL1, BL2, BL3, and BL4 are described. It should be emphasized that the descriptions and assignments of physical containment detailed below are based on existing approaches to containment of pathogenic organisms (see [Appendix G-III-A](#), *Footnotes and References of Appendix G*). The National Cancer Institute describes three levels for research on oncogenic viruses which roughly correspond to our BL2, BL3, and BL4 levels (see [Appendix G-III-C](#), *Footnotes and References of Appendix G*).

It is recognized that several different combinations of laboratory practices, containment equipment, and special laboratory design may be appropriate for containment of specific research activities. The *NIH Guidelines*, therefore, allow alternative selections of primary containment equipment within facilities that have been designed to provide BL3 and BL4 levels of physical containment. The selection of alternative methods of primary containment is dependent, however, on the level of biological containment provided by the host-vector system used in the experiment. Consideration will be given to other combinations which achieve an equivalent level of containment (see Sections [IV-C-1-b-\(1\)](#), *Major Actions* and [IV-C-1-b-\(2\)](#), *Minor Actions*).

Appendix G-II-A. Biosafety Level 1 (BL1) (See [Appendix G-III-M](#), *Footnotes and References of Appendix G*)

Appendix G-II-A-1. Standard Microbiological Practices (BL1)

Appendix G-II-A-1-a. Access to the laboratory is limited or restricted at the discretion of the Principal Investigator when experiments are in progress.

Appendix G-II-A-1-b. Work surfaces are decontaminated once a day and after any spill of viable material.

Appendix G-II-A-1-c. All contaminated liquid or solid wastes are decontaminated before disposal.

Appendix G-II-A-1-d. Mechanical pipetting devices are used; mouth pipetting is prohibited.

Appendix G-II-A-1-e. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only.

Appendix G-II-A-1-f. Persons wash their hands: (i) after they handle materials involving organisms containing recombinant DNA molecules and animals, and (ii) before exiting the laboratory.

Appendix G-II-A-1-g. All procedures are performed carefully to minimize the creation of aerosols.

Appendix G-II-A-1-h. In the interest of good personal hygiene, facilities (e.g., hand washing sink, shower, changing room) and protective clothing (e.g., uniforms, laboratory coats) shall be provided that are appropriate for the risk of exposure to viable organisms containing recombinant DNA molecules.

Appendix G-II-A-2. Special Practices (BL1)

Appendix G-II-A-2-a. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.

Appendix G-II-A-2-b. An insect and rodent control program is in effect.

Appendix G-II-A-3. Containment Equipment (BL1)

Appendix G-II-A-3-a. Special containment equipment is generally not required for manipulations of agents assigned to BL1.

Appendix G-II-A-4. Laboratory Facilities (BL1)

Appendix G-II-A-4-a. The laboratory is designed so that it can be easily cleaned.

Appendix G-II-A-4-b. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix G-II-A-4-c. Laboratory furniture is sturdy. Spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix G-II-A-4-d. Each laboratory contains a sink for hand washing.

Appendix G-II-A-4-e. If the laboratory has windows that open, they are fitted with fly screens.

Appendix G-II-B. Biosafety Level 2 (BL2) (See [Appendix G-III-N](#), *Footnotes and References of Appendix G*)**Appendix G-II-B-1. Standard Microbiological Practices (BL2)**

Appendix G-II-B-1-a. Access to the laboratory is limited or restricted by the Principal Investigator when work with organisms containing recombinant DNA molecules is in progress.

Appendix G-II-B-1-b. Work surfaces are decontaminated at least once a day and after any spill of viable material.

Appendix G-II-B-1-c. All contaminated liquid or solid wastes are decontaminated before disposal.

Appendix G-II-B-1-d. Mechanical pipetting devices are used; mouth pipetting is prohibited.

Appendix G-II-B-1-e. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only.

Appendix G-II-B-1-f. Persons wash their hands: (i) after handling materials involving organisms containing recombinant DNA molecules and animals, and (ii) when exiting the laboratory.

Appendix G-II-B-1-g. All procedures are performed carefully to minimize the creation of aerosols.

Appendix G-II-B-1-h. Experiments of lesser biohazard potential can be conducted concurrently in carefully demarcated areas of the same laboratory.

Appendix G-II-B-2. Special Practices (BL2)

Appendix G-II-B-2-a. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.

Appendix G-II-B-2-b. The Principal Investigator limits access to the laboratory. The Principal Investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

Appendix G-II-B-2-c. The Principal Investigator establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific entry requirements (e.g., immunization) may enter the laboratory or animal rooms.

Appendix G-II-B-2-d. When the organisms containing recombinant DNA molecules in use in the laboratory require special provisions for entry (e.g., vaccination), a hazard warning sign incorporating the universal biosafety symbol is posted on the access door to the laboratory work area. The hazard warning sign identifies the agent, lists the name and telephone number of the Principal Investigator or other responsible person(s), and indicates the special requirement(s) for entering the laboratory.

Appendix G-II-B-2-e. An insect and rodent control program is in effect.

Appendix G-II-B-2-f. Laboratory coats, gowns, smocks, or uniforms are worn while in the laboratory. Before exiting the laboratory for non-laboratory areas (e.g., cafeteria, library, administrative offices), this protective clothing is removed and left in the laboratory or covered with a clean coat not used in the laboratory.

Appendix G-II-B-2-g. Animals not involved in the work being performed are not permitted in the laboratory.

Appendix G-II-B-2-h. Special care is taken to avoid skin contamination with organisms containing recombinant DNA molecules; gloves should be worn when handling experimental animals and when skin contact with the agent is unavoidable.

Appendix G-II-B-2-i. All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

Appendix G-II-B-2-j. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably autoclaved, before discard or reuse.

Appendix G-II-B-2-k. Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Institutional Biosafety Committee and NIH/OBA. Reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

Appendix G-II-B-2-l. When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically depending on the agents handled or the function of the facility.

Appendix G-II-B-2-m. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

Appendix G-II-B-3. Containment Equipment (BL2)

Appendix G-II-B-3-a. Biological safety cabinets (Class I or II) (see [Appendix G-III-L, Footnotes and References of Appendix G](#)) or other appropriate personal protective or physical containment devices are used whenever:

Appendix G-II-B-3-a-(1). Procedures with a high potential for creating aerosols are conducted (see [Appendix G-III-O, Footnotes and References of Appendix G](#)). These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of materials whose internal pressures may be different from ambient pressures, intranasal inoculation of animals, and harvesting infected tissues from animals or eggs.

Appendix G-II-B-3-a-(2). High concentrations or large volumes of organisms containing recombinant DNA molecules are used. Such materials may be centrifuged in the open laboratory if sealed beads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

Appendix G-II-B-4. Laboratory Facilities (BL2)

Appendix G-II-B-4-a. The laboratory is designed so that it can be easily cleaned.

Appendix G-II-B-4-b. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix G-II-B-4-c. Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix G-II-B-4-d. Each laboratory contains a sink for hand washing.

Appendix G-II-B-4-e. If the laboratory has windows that open, they are fitted with fly screens.

Appendix G-II-B-4-f. An autoclave for decontaminating laboratory wastes is available.

Appendix G-II-C. Biosafety Level 3 (BL3) (See [Appendix G-III-P, Footnotes and References of Appendix G](#))

Appendix G-II-C-1. Standard Microbiological Practices (BL3)

Appendix G-II-C-1-a. Work surfaces are decontaminated at least once a day and after any spill of viable material.

Appendix G-II-C-1-b. All contaminated liquid or solid wastes are decontaminated before disposal.

Appendix G-II-C-1-c. Mechanical pipetting devices are used; mouth pipetting is prohibited.

Appendix G-II-C-1-d. Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the work area.

Appendix G-II-C-1-e. Persons wash their hands: (i) after handling materials involving organisms containing recombinant DNA molecules, and handling animals, and (ii) when exiting the laboratory.

Appendix G-II-C-1-f. All procedures are performed carefully to minimize the creation of aerosols.

Appendix G-II-C-1-g. Persons under 16 years of age shall not enter the laboratory.

Appendix G-II-C-1-h. If experiments involving other organisms which require lower levels of containment are to be conducted in the same laboratory concurrently with experiments requiring BL3 level physical containment, they shall be conducted in accordance with all BL3 level laboratory practices.

Appendix G-II-C-2. Special Practices (BL3)

Appendix G-II-C-2-a. Laboratory doors are kept closed when experiments are in progress.

Appendix G-II-C-2-b. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.

Appendix G-II-C-2-c. The Principal Investigator controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. The Principal Investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

Appendix G-II-C-2-d. The Principal Investigator establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures entering the laboratory or animal rooms.

Appendix G-II-C-2-e. When organisms containing recombinant DNA molecules or experimental animals are present in the laboratory or containment module, a hazard warning sign incorporating the universal biosafety symbol is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the Principal Investigator or other responsible person(s), and indicates any special requirements for entering the laboratory such as the need for immunizations, respirators, or other personal protective measures.

Appendix G-II-C-2-f. All activities involving organisms containing recombinant DNA molecules are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench.

Appendix G-II-C-2-g. The work surfaces of biological safety cabinets and other containment equipment are decontaminated when work with organisms containing recombinant DNA molecules is finished. Plastic-backed paper toweling used on non-perforated work surfaces within biological safety cabinets facilitates clean-up.

Appendix G-II-C-2-h. An insect and rodent program is in effect.

Appendix G-II-C-2-i. Laboratory clothing that protects street clothing (e.g., solid front or wrap-around gowns, scrub suits, coveralls) is worn in the laboratory. Laboratory clothing is not worn outside the laboratory, and it is decontaminated prior to laundering or disposal.

Appendix G-II-C-2-j. Special care is taken to avoid skin contamination with contaminated materials; gloves should be worn when handling infected animals and when skin contact with infectious materials is unavoidable.

Appendix G-II-C-2-k. Molded surgical masks or respirators are worn in rooms containing experimental animals.

Appendix G-II-C-2-l. Animals and plants not related to the work being conducted are not permitted in the laboratory.

Appendix G-II-C-2-m. Laboratory animals held in a BL3 area shall be housed in partial-containment caging systems, such as Horsfall units (see [Appendix G-III-K](#), *Footnotes and References of Appendix G*), open cages placed in ventilated enclosures, solid-wall and -bottom cages covered by filter bonnets or solid-wall and -bottom cages placed on holding racks equipped with ultraviolet in radiation lamps and reflectors.

Note: Conventional caging systems may be used provided that all personnel wear appropriate personal protective devices. These protective devices shall include at a minimum wrap-around gowns, head covers, gloves, shoe covers, and respirators. All personnel shall shower on exit from areas where these devices are required.

Appendix G-II-C-2-n. All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

Appendix G-II-C-2-o. Vacuum lines are protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.

Appendix G-II-C-2-p. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix G-II-C-2-q. Spills and accidents which result in overt or potential exposures to organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, and NIH/OBA. Reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained.

Appendix G-II-C-2-r. Baseline serum samples for all laboratory and other at-risk personnel should be collected and stored. Additional serum specimens may be collected periodically depending on the agents handled or the function of the laboratory.

Appendix G-II-C-2-s. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow the instructions on practices and procedures.

Appendix G-II-C-2-t. Alternative Selection of Containment Equipment (BL3)

Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified may be conducted in the BL3 laboratory using containment equipment specified for the BL2 level of physical containment. Experimental procedures involving a host-vector system that provides a one-step lower level of biological containment than that specified may be conducted in the BL3 laboratory using containment equipment specified for the BL4 level of physical containment. Alternative combination of containment safeguards are shown in [Appendix G-Table 1](#), *Possible Alternate Combinations of Physical and Biological Containment Safeguards*.

Appendix G-II-C-3. Containment Equipment (BL3)

Appendix G-II-C-3-a. Biological safety cabinets (Class I, II, or III) (see [Appendix G-III-L, Footnotes and References of Appendix G](#)) or other appropriate combinations of personal protective or physical containment devices (e.g., special protective clothing, masks, gloves, respirators, centrifuge safety cups, sealed centrifuge rotors, and containment caging for animals) are used for all activities with organisms containing recombinant DNA molecules which pose a threat of aerosol exposure. These include: manipulation of cultures and of those clinical or environmental materials which may be a source of aerosols; the aerosol challenge of experimental animals; the harvesting of infected tissues or fluids from experimental animals and embryonate eggs; and the necropsy of experimental animals.

Appendix G-II-C-4. Laboratory Facilities (BL3)

Appendix G-II-C-4-a. The laboratory is separated from areas which are open to unrestricted traffic flow within the building. Passage through two sets of doors is the basic requirement for entry into the laboratory from access corridors or other contiguous areas. Physical separation of the high containment laboratory from access corridors or other laboratories or activities may be provided by a double-doored clothes change room (showers may be included), airlock, or other access facility which requires passage through two sets of doors before entering the laboratory.

Appendix G-II-C-4-b. The interior surfaces of walls, floors, and ceilings are water resistant so that they can be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate decontaminating the area.

Appendix G-II-C-4-c. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix G-II-C-4-d. Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix G-II-C-4-e. Each laboratory contains a sink for hand washing. The sink is foot, elbow, or automatically operated and is located near the laboratory exit door.

Appendix G-II-C-4-f. Windows in the laboratory are closed and sealed.

Appendix G-II-C-4-g. Access doors to the laboratory or containment module are self-closing.

Appendix G-II-C-4-h. An autoclave for decontaminating laboratory wastes is available preferably within the laboratory.

Appendix G-II-C-4-i. A ducted exhaust air ventilation system is provided. This system creates directional airflow that draws air into the laboratory through the entry area. The exhaust air is not recirculated to any other area of the building, is discharged to the outside, and is dispersed away from the occupied areas and air intakes. Personnel shall verify that the direction of the airflow (into the laboratory) is proper. The exhaust air from the laboratory room may be discharged to the outside without being filtered or otherwise treated.

Appendix G-II-C-4-j. The high efficiency particulate air/HEPA filtered exhaust air from Class I or Class II biological safety cabinets is discharged directly to the outside or through the building exhaust system. Exhaust air from Class I or II biological safety cabinets may be recirculated within the laboratory if the cabinet is tested and certified at least every twelve months. If the HEPA-filtered exhaust air from Class I or II biological safety cabinets is to be discharged to the outside through the building exhaust air system, it is connected to this system in a manner (e.g., thimble unit connection (see [Appendix G-III-L, Footnotes and References of Appendix G](#))) that avoids any interference with the air balance of the cabinets or building exhaust system.

Appendix G-II-C-5. Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses.**Appendix G-II-C-5-a. Containment, Practices, and Training for Research with Risk Group 3 Influenza Viruses (BL3 Enhanced).**

Appendix G-II-C-5-a-(1). In addition to standard BL3 practices, the following additional personal protective equipment and practices shall be used: (1) Powered Air-purifying Respirators (PAPR) are worn. (2) Street clothes are changed to protective suit (e.g., wrap-back disposable gown, olefin protective suit). (3) Double gloves are worn. (4) Appropriate shoe coverings are worn (e.g., double disposable shoe coverings, single disposable shoe coverings if worn with footwear dedicated to BL3 enhanced laboratory use, or impervious boots or shoes of rubber or other suitable material that can be decontaminated). (5) Showers prior to exiting the laboratory should be considered depending on risk assessment of research activities.

Appendix G-II-C-5-a-(2). As proper training of laboratory workers is an essential component of biosafety, retraining and periodic reassessments (at least annually) in BL3 enhanced practices, especially the proper use of respiratory equipment, such as PAPRs, and clothing changes is required.

Appendix G-II-C-5-a-(3). Reporting of all spills and accidents, even if relatively minor, is required as described in Appendix G-II-C-2-q.

Appendix G-II-C-5-a-(4). To avoid inadvertent cross contamination of 1918 H1N1, HPAI H5N1 or human H2N2 (1957-1968): (1) Containment facilities and practices appropriate for highest risk group virus shall be used at all times with lower risk group viruses, when studied in the same laboratory room. (2) Tissue cultures with these viruses shall be conducted at separate times (temporal spacing) in the same room. (3) Separate reagents shall be used to minimize risk of cross contamination. (4) A laboratory worker shall not perform concurrent influenza virus experiments that carry the risk of unintended reassortment among 1918 H1N1, human H2N2 (1957-1968), HPAI H5N1 and other human influenza viruses. (5) Two or more laboratory workers shall not perform within the same work area simultaneous influenza virus experiments that carry the risk of unintended segment reassortment between 1918 H1N1, or HPAI H5N1, or human H2N2 (1957-1968) and other human influenza viruses. (6) Between experiments good biosafety decontamination practices (e.g., surface and biosafety cabinet surface decontamination according to standard BL3 procedures) shall be used and there shall be a thirty minute wait period after decontamination before equipment is used for experiments with any other influenza A viruses. (7) Between experiments, in addition to decontamination of the work area, clothing changes and PAPR disinfection shall be performed prior to handling a different influenza virus in the same work area. (Shower-out capability may be required by [USDA/APHIS](#) for certain experiments with HPAI H5N1.)

Appendix G-II-C-5-a-(5). Continued susceptibility of the reassortant influenza viruses containing genes and/or segments from 1918 H1N1, HPAI H5N1, and human H2N2 (1957-1968) to antiviral agents shall be established by sequence analysis or suitable biological assays. After manipulation of genes that influence sensitivity to antiviral agents, susceptibility to these agents shall be reconfirmed.

Appendix G-II-C-5-b. Containment for Animal Research.

Guidance provided in [Appendix G-II-C](#) and [Appendix Q-II-C](#) is applicable with the following emphasis on standard BL3 or BL3-N containment or additional enhancements.

Appendix G-II-C-5-b-(1). Research with small animals shall be conducted in a class II biosafety cabinet. Small animals such as rodents (e.g. mice, hamsters, rats, guinea pigs) can be housed within a negative pressure BL3 animal suite using high-density individually vented caging (IVC) systems that independently supply high efficiency particulate air/HEPA-filtered and directional air circulation. Other animals (e.g. rabbits, ferrets) that are of a size or have growth or caging requirements that preclude the use of high-density IVC systems are to be housed in negative pressure bioisolators.

Appendix G-II-C-5-b-(2). Large animals such as non-human primates shall be housed in primary barrier environments according to BL3-N containment requirements (see [Appendix Q-II-c](#)).

Appendix G-II-C-5-b-(3). Specialized training and proven competency in all assigned practices and procedures shall be required for laboratory staff, including staff involved in animal care.

Appendix G-II-C-5-b-(4). For HPAI H5N1 research, the *NIH Guidelines* defer to [USDA/APHIS](#) recommendations for biocontainment practices for loose housed animals.

Appendix G-II-C-5-c. Occupational Health

A detailed occupational health plan shall be developed in advance of working with these agents in consultation, as needed, with individuals with the appropriate clinical expertise. In addition, the appropriate public health authority shall be consulted (e.g. local public health officials) on the plan and a mock drill of this plan shall be undertaken periodically. The plan should include an incident reporting system and laboratory workers shall report all incidents.

Appendix G-II-C-5-c-(1). Laboratory workers shall be provided with medical cards which include, at a minimum, the following information: characterization of the influenza virus to which they have been potentially exposed, and 24-hour contact numbers for the principal investigator and institution's occupational health care provider(s).

Appendix G-II-C-5-c-(2). A detailed occupational health plan shall include: (1) Unless there is a medical contraindication to vaccination (e.g. severe egg allergy) annual seasonal influenza vaccination as prerequisite for research to reduce risk of influenza like illness requiring isolation and tests to rule out infection with experimental virus and possible co-infection with circulating influenza strains. (2) Virus specific vaccination, if available, should be offered. (3) Reporting of all respiratory symptoms and/or fever (i.e. influenza-like illnesses). (4) 24-hour access to a medical facility that is prepared to implement appropriate respiratory isolation to prevent transmission and is able to provide appropriate antiviral agents. Real-time reverse transcription-polymerase chain reaction (RT-PCR) procedures should be used to discriminate these viruses from currently circulating human influenza viruses. For exposures to viruses containing genes from 1918 H1N1 or the HA gene from human H2N2 (1957-1968), specimens shall be sent to the [CDC](#) for testing (RT-PCR and confirmatory sequencing).

Appendix G-II-C-5-c-(3). In preparing to perform research with 1918 H1N1, human H2N2 (1957-1968), or HPAI H5N1, principal investigators should develop a clear plan specifying who will be contacted in the event of a potential exposure (during and after work hours) to conduct a risk assessment and make decisions as to the required response, including the need for and extent of isolation of the exposed worker. After any kind of potential exposure, a rapid risk assessment shall be performed by the principal investigator, health and biosafety officials and subsequent actions should depend on the appraised level of risk of respiratory infection for the individual and potential for transmission to others. A laboratory worker performing research with either an influenza virus containing the HA gene from human H2N2 or an influenza virus containing genes and/or segments from 1918 H1N1, shall be informed in advance that, in the case of a *known* laboratory exposure with a high risk for infection, e.g., involving the upper or lower respiratory tract or mucous membranes, the laboratory worker will need to be isolated in a predetermined facility, rather than home isolation, until infection can be ruled out by testing (e.g., negative RT-PCR for 1918 H1N1 or human H2N2 (1957-1968)) of appropriately timed specimens. Laboratory workers shall be informed in advance that in the case of a known laboratory exposure to highly pathogenic avian influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage with high risk for infection, they should be prepared to self isolate (for example at home) until infection can be ruled out by testing (e.g., negative RT-PCR for HPAI H5N1) of appropriately timed specimens. The action taken for other types of exposures should be based on the risk assessment. In addition, based on the risk assessment: (1) treatment with appropriate antiviral agents shall be initiated, and (2) the appropriate public health authorities shall be notified.

Appendix G-II-C-5-c-(4). Influenza-like illness. If a laboratory worker, who had recent exposure (within ten days) to influenza viruses containing the human H2N2 HA gene or any gene from the 1918 H1N1 or HPAI H5N1 viruses, or to animals exposed to such viruses, demonstrates symptoms and/or signs of influenza infection (e.g., fever/chills, cough, myalgias, headache), then the lab worker shall report by phone to the supervisor/principal investigator and other individuals identified in the occupational health plan. The laboratory worker shall be transported to a healthcare facility that can provide adequate respiratory isolation, appropriate medical therapy, and testing to determine whether the infection is due to a recombinant influenza virus. The appropriate public health authorities shall be informed whenever a suspected case is isolated.

Appendix G-II-C-5-c-(5). For 1918 H1N1 research, the use of antiviral agents (e.g., oseltamivir) for pre-exposure prophylaxis shall be discussed with laboratory workers in advance including a discussion of the data on the safety of long term exposure to these agents and their ability to reduce the risk of clinical disease and the limits of the data regarding protection of close contacts and the community.

Appendix G-II-C-5-c-(6). Antiviral agents for post-exposure prophylaxis shall be provided only after medical evaluation. Home supplies shall not be provided in advance for research with 1918 H1N1 or influenza viruses containing the HA gene from human H2N2.

Appendix G-II-D. Biosafety Level 4 (BL4)

Appendix G-II-D-1. Standard Microbiological Practices (BL4)

Appendix G-II-D-1-a. Work surfaces are decontaminated at least once a day and immediately after any spill of viable material.

Appendix G-II-D-1-b. Only mechanical pipetting devices are used.

Appendix G-II-D-1-c. Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the laboratory.

Appendix G-II-D-1-d. All procedures are performed carefully to minimize the creation of aerosols.

Appendix G-II-D-2. Special Practices (BL4)

Appendix G-II-D-2-a. Biological materials to be removed from the Class III cabinets or from the maximum containment laboratory in a viable or intact state are transferred to a non-breakable, sealed primary container and then enclosed in a non-breakable, sealed secondary container which is removed from the facility through a disinfectant dunk tank, fumigation chamber, or an airlock designed for this purpose.

Appendix G-II-D-2-b. No materials, except for biological materials that are to remain in a viable or intact state, are removed from the maximum containment laboratory unless they have been autoclaved or decontaminated before exiting the facility. Equipment or material which might be damaged by high temperatures or steam is decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

Appendix G-II-D-2-c. Only persons whose presence in the facility or individual laboratory rooms is required for program or support purposes are authorized to enter. The supervisor has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. Access to the facility is limited by means of secure, locked doors; accessibility is managed by the Principal Investigator, Biological Safety Officer, or other person responsible for the physical security of the facility. Before entering, persons are advised of the potential biohazards and instructed as to appropriate safeguards for ensuring their safety. Authorized persons comply with the instructions and all other applicable entry and exit procedures. A logbook signed by all personnel indicates the date and time of each entry and exit. Practical and effective protocols for emergency situations are established.

Appendix G-II-D-2-d. Personnel enter and exit the facility only through the clothing change and shower rooms. Personnel shower each time they exit the facility. Personnel use the air locks to enter or exit the laboratory only in an emergency.

Appendix G-II-D-2-e. Street clothing is removed in the outer clothing change room and kept there. Complete laboratory clothing (may be disposable), including undergarments, pants and shirts or jump suits, shoes, and gloves, is provided and used by all personnel entering the facility. Head covers are provided for personnel who do not wash their hair during the exit shower. When exiting the laboratory and before proceeding into the shower area, personnel remove their laboratory clothing and store it in a locker or hamper in the inner change room. Protective clothing shall be decontaminated prior to laundering or disposal.

Appendix G-II-D-2-f. When materials that contain organisms containing recombinant DNA molecules or experimental animals are present in the laboratory or animal rooms, a hazard warning sign incorporating the universal biosafety symbol is posted on all access doors. The sign identifies the agent, lists the name of the Principal Investigator or other responsible person(s), and indicates any special requirements for entering the area (e.g., the need for immunizations or respirators).

Appendix G-II-D-2-g. Supplies and materials needed in the facility are brought in by way of the double-doored autoclave, fumigation chamber, or airlock which is appropriately decontaminated between each use. After securing the outer doors, personnel within the facility retrieve the materials by opening the interior doors or the autoclave, fumigation chamber, or airlock. These doors are secured after materials are brought into the facility.

Appendix G-II-D-2-h. An insect and rodent control program is in effect.

Appendix G-II-D-2-i. Materials (e.g., plants, animals, and clothing) not related to the experiment being conducted are not permitted in the facility.

Appendix G-II-D-2-j. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral part of unit) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be placed in a puncture-resistant container and decontaminated, preferably by autoclaving before discard or reuse. Whenever possible, cannulas are used instead of sharp needles (e.g., gavage).

Appendix G-II-D-2-k. A system is set up for reporting laboratory accidents, exposures, employee absenteeism, and for the medical surveillance of potential laboratory-associated illnesses. Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, and NIH/OBA. Reports to the NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Written records are prepared and maintained. An essential adjunct to such a reporting-surveillance system is the availability of a facility for quarantine, isolation, and medical care of personnel with potential or known laboratory associated illnesses.

Appendix G-II-D-2-l. Laboratory animals involved in experiments requiring BL4 level physical containment shall be housed either in cages contained in Class III cabinets or in partial containment caging systems, such as Horsfall units (see [Appendix G-III-K](#), *Footnotes and References of Appendix G*), open cages placed in ventilated enclosures, or solid-wall and -bottom cages placed on holding racks equipped with ultraviolet irradiation lamps and reflectors that are located in a specially designed area in which all personnel are required to wear one-piece positive pressure suits.

Appendix G-II-D-2-m. Alternative Selection of Containment Equipment (BL4)

Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified may be conducted in the BL4 facility using containment equipment requirements specified for the BL3 level of physical containment. Alternative combinations of containment safeguards are shown in Appendix G-Table 1, *Possible Alternate Combinations of Physical and Biological Containment Safeguards*.

Appendix G-II-D-3. Containment Equipment (BL4)

Appendix G-II-D-3-a. All procedures within the facility with agents assigned to Biosafety Level 4 are conducted in the Class III biological safety cabinet or in Class I or II biological safety cabinets used in conjunction with one-piece positive pressure personnel suits ventilated by a life-support system.

Appendix G-II-D-4. Laboratory Facilities (BL4)

Appendix G-II-D-4-a. The maximum containment facility consists of either a separate building or a clearly demarcated and isolated zone within a building. Outer and inner change rooms separated by a shower are provided for personnel entering and exiting the facility. A double-doored autoclave, fumigation chamber, or ventilated airlock is provided for passage of those materials, supplies, or equipment which are not brought into the facility through the change room.

Appendix G-II-D-4-b. Walls, floors, and ceilings of the facility are constructed to form a sealed internal shell which facilitates fumigation and is animal and insect proof. The internal surfaces of this shell are resistant to liquids and chemicals, thus facilitating cleaning and decontamination of the area. All penetrations in these structures and surfaces are sealed. Any drains in the floors contain traps filled with a chemical disinfectant of demonstrated efficacy against the target agent, and they are connected directly to the liquid waste decontamination system. Sewer and other ventilation lines contain high efficiency particulate air/HEPA filters.

Appendix G-II-D-4-c. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize the horizontal surface area on which dust can settle.

Appendix G-II-D-4-d. Bench tops have seamless surfaces which are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix G-II-D-4-e. Laboratory furniture is simple and of sturdy construction; and spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix G-II-D-4-f. A foot, elbow, or automatically operated hand washing sink is provided near the door of each laboratory room in the facility.

Appendix G-II-D-4-g. If there is a central vacuum system, it does not serve areas outside the facility. In-line high efficiency particulate air/HEPA filters are placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement. Other liquid and gas services to the facility are protected by devices that prevent back-flow.

Appendix G-II-D-4-h. If water fountains are provided, they are foot operated and are located in the facility corridors outside the laboratory. The water service to the fountain is not connected to the back-flow protected distribution system supplying water to the laboratory areas.

Appendix G-II-D-4-i. Access doors to the laboratory are self-closing and locking.

Appendix G-II-D-4-j. Any windows are breakage resistant.

Appendix G-II-D-4-k. A double-doored autoclave is provided for decontaminating materials passing out of the facility. The autoclave door which opens to the area external to the facility is sealed to the outer wall and automatically controlled so that the outside door can only be opened after the autoclave "sterilization" cycle has been completed.

Appendix G-II-D-4-l. A pass-through dunk tank, fumigation chamber, or an equivalent decontamination method is provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the facility.

Appendix G-II-D-4-m. Liquid effluent from laboratory sinks, biological safety cabinets, floors, and autoclave chambers are decontaminated by heat treatment before being released from the maximum containment facility. Liquid wastes from shower rooms and toilets may be decontaminated with chemical disinfectants or by heat in the liquid waste decontamination system. The procedure used for heat decontamination of liquid wastes is evaluated mechanically and biologically by using a recording thermometer and an indicator microorganism with a defined heat susceptibility pattern. If liquid wastes from the shower room are decontaminated with chemical disinfectants, the chemical used is of demonstrated efficacy against the target or indicator microorganisms.

Appendix G-II-D-4-n. An individual supply and exhaust air ventilation system is provided. The system maintains pressure differentials and directional airflow as required to assure flows inward from areas outside of the facility toward areas of highest potential risk within the facility. Manometers are used to sense pressure differentials between adjacent areas maintained at different pressure levels. If a system malfunctions, the manometers sound an alarm. The supply and exhaust airflow is interlocked to assure inward (or zero) airflow at all times.

Appendix G-II-D-4-o. The exhaust air from the facility is filtered through high efficiency particulate air/HEPA filters and discharged to the outside so that it is dispersed away from occupied buildings and air intakes. Within the facility, the filters are located as near the laboratories as practicable in order to reduce the length of potentially contaminated air ducts. The filter chambers are designed to allow *in situ* decontamination before filters are removed and to facilitate certification testing after they are replaced. Coarse filters and HEPA filters are provided to treat air supplied to the facility in order to increase the lifetime of the exhaust HEPA filters and to protect the supply air system should air pressures become unbalanced in the laboratory.

Appendix G-II-D-4-p. The treated exhaust air from Class I and II biological safety cabinets may be discharged into the laboratory room environment or the outside through the facility air exhaust system. If exhaust air from Class I or II biological safety cabinets is discharged into the laboratory the cabinets are tested and certified at six-month intervals. *The exhaust air from Class III biological safety cabinets is discharged, without recirculation through two sets of high efficiency particulate air/HEPA filters in series, via the facility exhaust air system.* If the treated exhaust air from any of these cabinets is discharged to the outside through the facility exhaust air system, it is connected to this system in a manner (e.g., thimble unit connection (see [Appendix G-III-L, Footnotes and References of Appendix G](#))) that avoids any interference with the air balance of the cabinets or the facility exhaust air system.

Appendix G-II-D-4-q. A specially designed suit area may be provided in the facility. Personnel who enter this area shall wear a one-piece positive pressure suit that is ventilated by a life-support system. The life-support system includes alarms and emergency backup breathing air tanks. Entry to this area is through an airlock fitted with airtight doors. A chemical shower is provided to decontaminate the surface of the suit before the worker exits the area. The exhaust air from the suit area is filtered by two sets of high efficiency particulate air/HEPA filters installed in series. A duplicate filtration unit, exhaust fan, and an automatically starting emergency power source are provided. The air pressure within the suit area is greater than that of any adjacent area. Emergency lighting and communication systems are provided. All penetrations into the internal shell of the suit are sealed. A double-doored autoclave is provided for decontaminating waste materials to be removed from the suit areas.

Appendix G - Table 1. Possible Alternate Combinations Of Physical And Biological Containment Safeguards

Classification of Physical & Biological Containment	Alternate Physical Containment			Alternate Biological Containment
	Laboratory Facilities	Laboratory Practices	Laboratory Equipment	
BL3/HV2	BL3 BL3	BL3 BL3	BL3 BL4	HV2 HV1
BL3/HV1	BL3 BL3	BL3 BL3	BL3 BL2	HV1 HV2
BL4/HV1	BL4 BL4	BL4 BL4	BL4 BL3	HV1 HV2

BL - Biosafety Level

HV - Host-Vector System

Appendix G-III. Footnotes and References of Appendix G

Appendix G-III-A. *Biosafety in Microbiological and Biomedical Laboratories*, 5th edition, 2007, DHHS, Public Health Service, Centers for Disease Control and Prevention, Atlanta, Georgia, and National Institutes of Health, Bethesda, Maryland.

Appendix G-III-B. *Biosafety in Microbiological and Biomedical Laboratories*, 3rd edition, May 1993, U.S. DHHS, Public Health Service, Centers for Disease Control and Prevention, Atlanta, Georgia, and NIH, Bethesda, Maryland.

Appendix G-III-C. *National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses*, U.S. Department of Health, Education, and Welfare Publication No. (NIH) 75-790, October 1974.

Appendix G-III-D. *National Institutes of Health Biohazards Safety Guide*, U.S. Department of Health, Education, and Welfare, Public Health Service, NIH, U.S. Government Printing Office, Stock No. 1740-00383, 1974.

Appendix G-III-E. A. Hellman, M. N. Oxman, and R. Pollack (eds.), *Biohazards in Biological Research*, Cold Spring Harbor Laboratory 1973.

Appendix G-III-F. N. V. Steere (ed.), *Handbook of Laboratory Safety*, 2nd edition, The Chemical Rubber Co., Cleveland, Ohio, 1971.

Appendix G-III-G. Bodily, J. L., "General Administration of the Laboratory," H. L. Bodily, E. L. Updyke, and J. O. Mason (eds.), *Diagnostic Procedures for Bacterial, Mycotic, and Parasitic Infections*, American Public Health Association, New York, 1970, pp. 11-28.

Appendix G-III-H. Darlow, H. M. (1969). "Safety in the Microbiological Laboratory," in J. R. Norris and D. W. Robbins (eds.), *Methods in Microbiology*, Academic Press, Inc., New York, pp. 169-204.

Appendix G-III-I. *The Prevention of Laboratory Acquired Infection*, C. H. Collins, E. G. Hartley, and R. Pilsworth, Public Health Laboratory Service, Monograph Series No. 6, 1974.

Appendix G-III-J. Chatigny, M. A., "Protection Against Infection in the Microbiological Laboratory: Devices and Procedures," in W. W. Umbreit (ed.), *Advances in Applied Microbiology*, Academic Press, New York, New York, 1961, 3:131-192.

Appendix G-III-K. Horsfall, F. L. Jr., and J. H. Baner, *Individual Isolation of Infected Animals in a Single Room*, J. Bact., 1940, 40, 569-580.

Appendix G-III-L. Biological safety cabinets referred to in this section are classified as Class I, Class II, or Class III cabinets. A Class I is a ventilated cabinet for personnel protection having an inward flow of air away from the operator. The exhaust air from this cabinet is filtered through a high efficiency particulate air/HEPA filter. This cabinet is used in three operational modes: (i) with a full-width open front, (ii) with an installed front closure panel (having four 6-inch diameter openings) without gloves, and (iii) with an installed front closure panel equipped with arm-length rubber gloves. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater. A Class II cabinet is a ventilated cabinet for personnel and product protection having an open front with inward air flow for personnel protection, and HEPA filtered mass recirculated air flow for product protection. The cabinet exhaust air is filtered through a HEPA filter. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater. Design and performance specifications for Class II cabinets have been adopted by the National Sanitation Foundation, Ann Arbor, Michigan. A Class III cabinet is a closed-front ventilated cabinet of gas tight construction which provides the highest level of personnel protection of all biosafety safety cabinets. The interior of the cabinet is protected from contaminants exterior to the cabinet. The cabinet is fitted with arm-length rubber gloves and is operated under a negative pressure of at least 0.5 inches water gauge. All supply air is filtered through HEPA filters. Exhaust air is filtered through two HEPA filters or one HEPA filter and incinerator before being discharged to the outside environment. National Sanitation Foundation Standard 49. 1976. Class II (Laminar Flow) Biohazard Cabinetry, Ann Arbor, Michigan.

Appendix G-III-M. Biosafety Level 1 is suitable for work involving agents of unknown or minimal potential hazard to laboratory personnel and the environment. The laboratory is not separated from the general traffic patterns in the building. Work is generally conducted on open bench tops. Special containment equipment is not required or generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science (see [Appendix G-III-A, Footnotes and References of Appendix G](#)).

Appendix G-III-N. Biosafety Level 2 is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that: (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists; (2) access to the laboratory is limited when work is being conducted; and (3) certain procedures in which infectious aerosols are created are conducted in biological safety cabinets or other physical containment equipment (see [Appendix G-III-A, Footnotes and References of Appendix G](#)).

Appendix G-III-O. Office of Research Safety, National Cancer Institute, and the Special Committee of Safety and Health Experts, *Laboratory Safety Monograph: A Supplement to the NIH Guidelines for Recombinant DNA Research*, NIH, Bethesda, Maryland 1978.

Appendix G-III-P. Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is conducted with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents and are supervised by competent scientists who are experienced in working with these agents. All procedures involving the manipulation of infectious material are conducted within biological safety cabinets or other physical containment devices or by personnel wearing appropriate personal protective clothing and devices. The laboratory has special engineering and design features. It is recognized, however, that many existing facilities may not have all the facility safeguards recommended for BL3 (e.g., access zone, sealed penetrations, and directional airflow, etc.). In these circumstances, acceptable safety may be achieved for routine or repetitive operations (e.g., diagnostic procedures involving the propagation of an agent for identification, typing, and susceptibility testing) in laboratories where facility features satisfy BL2 recommendations provided the recommended "Standard Microbiological Practices," "Special Practices," and "Containment Equipment" for BL3 are rigorously followed. The decision to implement this modification of BL3 recommendations should be made only by the Principal Investigator.

APPENDIX H. SHIPMENT

Recombinant DNA molecules contained in an organism or in a viral genome shall be shipped under the applicable regulations of the U.S. Postal Service (39 Code of Federal Regulations, Part 3); the Public Health Service (42 Code of Federal Regulations, Part 72); the U.S. Department of Agriculture (9 Code of Federal Regulations, Subchapters D and E; 7 CFR, Part 340); and/or the U.S. Department of Transportation (49 Code of Federal Regulations, Parts 171-179).

Note. A host-vector system may be proposed for certification by the NIH Director in accordance with the procedures set forth in Appendix I-II, *Certification of Host-Vector Systems*. In order to ensure protection for proprietary data, any public notice regarding a host-vector system which is designated by the institution as proprietary under [Section IV-D, Voluntary Compliance](#), will be issued only after consultation with the institution as to the content of the notice (see [Section IV-D-3, Certification of Host-Vector Systems - Voluntary Compliance](#)).

Appendix H-I. Host organisms or viruses will be shipped as etiologic agents, regardless of whether they contain recombinant DNA, if they are regulated as human pathogens by the Public Health Service (42 Code of Federal Regulations, Part 72) or as animal pathogens or plant pests under the U.S. Department of Agriculture, Animal and Plant Health Inspection Service (Titles 9 and 7 Code of Federal Regulations, respectively).

Appendix H-II. Host organisms and viruses will be shipped as etiologic agents if they contain recombinant DNA when: (i) the recombinant DNA includes the complete genome of a host organism or virus regulated as a human or animal pathogen or a plant pest; or (ii) the recombinant DNA codes for a toxin or other factor directly involved in eliciting human, animal, or plant disease or inhibiting plant growth, and is carried on an expression vector or within the host chromosome and/or when the host organism contains a conjugation proficient plasmid or a generalized transducing phage; or (iii) the recombinant DNA comes from a host organism or virus regulated as a human or animal pathogen or as a plant pest and has not been adequately characterized to demonstrate that it does not code for a factor involved in eliciting human, animal, or plant disease.

Appendix H-III. Footnotes and References of Appendix H

For further information on shipping etiologic agents contact: (i) The [Centers for Disease Control and Prevention](#), ATTN: Biohazards Control Office, 1600 Clifton Road, Atlanta, Georgia 30333, (404) 639-3883, FTS 236-3883; (ii) The U.S. Department of Transportation, ATTN: Office of Hazardous Materials Transportation, 400 7th Street, S.W., Washington, DC 20590, (202) 366-4545; or (iii) U.S. Department of Agriculture, ATTN: [Animal and Plant Health Inspection Service \(APHIS\)](#), Veterinary Services, National Center for Import-Export, Products Program, 4700 River Road, Unit 40, Riverdale, Maryland 20737. Phone: (301) 734-8499; Fax: (301) 734-8226.

APPENDIX I. BIOLOGICAL CONTAINMENT (See [Appendix E](#), *Certified Host-Vector Systems*)

Appendix I-I. Levels of Biological Containment

In consideration of biological containment, the vector (plasmid, organelle, or virus) for the recombinant DNA and the host (bacterial, plant, or animal cell) in which the vector is propagated in the laboratory will be considered together. Any combination of vector and host which is to provide biological containment shall be chosen or constructed so that the following types of "escape" are minimized: (i) survival of the vector in its host outside the laboratory, and (ii) transmission of the vector from the propagation host to other non-laboratory hosts. The following levels of biological containment (host-vector systems) for prokaryotes are established. Appendices I-I-A through I-II-B describe levels of biological containment (host-vector systems) for prokaryotes. Specific criteria will depend on the organisms to be used.

Appendix I-I-A. Host-Vector 1 Systems

Host-Vector 1 systems provide a moderate level of containment. Specific Host-Vector 1 systems are:

Appendix I-I-A-1. *Escherichia coli* K-12 Host-Vector 1 Systems (EK1)

The host is always *Escherichia coli* K-12 or a derivative thereof, and the vectors include non-conjugative plasmids (e.g., pSC101, Co1E1, or derivatives thereof (see Appendices [I-III-A](#) through [G](#), *Footnotes and References of Appendix I*) and variants of bacteriophage, such as lambda (see Appendices [I-III-H](#) through [O](#), *Footnotes and References of Appendix I*). The *Escherichia coli* K-12 hosts shall not contain conjugation-proficient plasmids, whether autonomous or integrated, or generalized transducing phages.

Appendix I-I-A-2. Other Host-Vector 1 Systems

At a minimum, hosts and vectors shall be comparable in containment to *Escherichia coli* K-12 with a non-conjugative plasmid or bacteriophage vector. [Appendix I-II](#), *Certification of Host-Vector Systems*, describes the data to be considered and mechanism for approval of Host-Vector 1 systems.

Appendix I-I-B. Host-Vector 2 Systems (EK2)

Host-Vector 2 Systems provide a high level of biological containment as demonstrated by data from suitable tests performed in the laboratory. Escape of the recombinant DNA either via survival of the organisms or via transmission of recombinant DNA to other organisms should be $< 1/10^8$ under specified conditions. Specific Host-Vector 2 systems are:

Appendix I-I-B-1. For *Escherichia coli* K-12 Host-Vector 2 systems (EK2) in which the vector is a plasmid, no more than $1/10^8$ host cells shall perpetuate a cloned DNA fragment under the specified non-permissive laboratory conditions designed to represent the natural environment, either by survival of the original host or as a consequence of transmission of the cloned DNA fragment.

Appendix I-I-B-2. For *Escherichia coli* K-12 Host-Vector 2 systems (EK2) in which the vector is a phage, no more than $1/10^8$ phage particles shall perpetuate a cloned DNA fragment under the specified non-permissive laboratory conditions designed to represent the natural environment, either as a prophage (in the inserted or plasmid form) in the laboratory host used for phage propagation, or survival in natural environments and transferring a cloned DNA fragment to other hosts (or their resident prophages).

Appendix I-II. Certification of Host-Vector Systems

Appendix I-II-A. Responsibility

Host-Vector 1 systems (other than *Escherichia coli* K-12) and Host-Vector 2 systems may not be designated as such until they have been certified by the NIH Director. Requests for certification of host-vector systems may be submitted to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Proposed host-vector systems will be reviewed by the RAC (see [Section IV-C-1-b-\(1\)-\(f\)](#), *Major Actions*). Initial review will be based on the construction, properties, and testing of the proposed host-vector system by a subcommittee composed of one or more RAC members and/or *ad hoc* experts. The RAC will evaluate the subcommittee's report and any other available information at the next scheduled RAC meeting. The NIH Director is responsible for certification of host-vector systems, following advice of the RAC. Minor modifications to existing host-vector systems (i.e., those that are of minimal or no consequence to the properties relevant to containment) may be certified by the NIH Director without prior RAC review (see [Section IV-C-1-b-\(2\)-\(f\)](#), *Minor Actions*). Once a host-vector system has been certified by the NIH Director, a notice of certification will be sent by NIH/OBA to the applicant and to the Institutional Biosafety Committee Chairs. A list of all currently certified host-vector systems is available from the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). The NIH Director may rescind the certification of a host-vector system (see [Section IV-C-1-b-\(2\)-\(g\)](#), *Minor Actions*). If certification is rescinded, NIH will instruct investigators to transfer cloned DNA into a different system or use the clones at a higher level of physical containment level, unless NIH determines that the already constructed clones incorporate adequate biological containment. Certification of an host-vector system does not extend to modifications of either the host or vector component of that system. Such modified systems shall be independently certified by the NIH Director. If modifications are minor, it may only be necessary for the investigator to submit data showing that the modifications have either improved or not impaired the major phenotypic traits on which the containment of the system depends. Substantial modifications to a certified host-vector system requires submission of complete testing data.

Appendix I-II-B. Data to be Submitted for Certification

Appendix I-II-B-1. Host-Vector 1 Systems Other than *Escherichia coli* K-12

The following types of data shall be submitted, modified as appropriate for the particular system under consideration: (i) a description of the organism and vector; the strain's natural habitat and growth requirements; its physiological properties, particularly those related to its reproduction, survival, and the mechanisms by which it exchanges genetic information; the range of organisms with which this organism normally exchanges genetic information and the type of information is exchanged; and any relevant information about its pathogenicity or toxicity; (ii) a description of the history of the particular strains and vectors to be used, including data on any mutations which render this organism less able to survive or transmit genetic information; and (iii) a general description of the range of experiments contemplated with emphasis on the need for developing such an Host-Vector 1 system.

Appendix I-II-B-2. Host-Vector 2 Systems

Investigators planning to request Host-Vector 2 systems certification may obtain instructions from NIH/OBA concerning data to be submitted (see [Appendices I-III-N and O](#), *Footnotes and References of Appendix I*). In general, the following types of data are required: (i) description of construction steps with indication of source, properties, and manner of introduction of genetic traits; (ii) quantitative data on the stability of genetic traits that contribute to the containment of the system; (iii) data on the survival of the host-vector system under non-permissive laboratory conditions designed to represent the relevant natural environment; (iv) data on transmissibility of the vector and/or a cloned DNA fragment under both permissive and non-permissive conditions; (v) data on all other properties of the system which affect containment and utility, including information on yields of phage or plasmid molecules, ease of DNA isolation, and ease of transfection or transformation; and (vi) in some cases, the investigator may be asked to submit data on survival and vector transmissibility from experiments in which the host-vector is fed to laboratory animals or one or more human subjects. Such *in vivo* data may be required to confirm the validity of predicting *in vivo* survival on the basis of *in vitro* experiments. Data shall be submitted 12 weeks prior to the RAC meeting at which such data will be considered by the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Investigators are encouraged to publish their data on the construction, properties, and testing of proposed Host Vector 2 systems prior to consideration of the system by the RAC and its subcommittee. Specific instructions concerning the submission of data for proposed *Escherichia coli* K-12 Host-Vector 2 system (EK2) involving either plasmids or bacteriophage in *Escherichia coli* K-12, are available from the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

Appendix I-III. Footnotes and References of Appendix I

Appendix I-III-A. Hersfield, V., H. W. Boyer, C. Yanofsky, M. A. Lovett, and D. R. Helinski, *Plasmid Co1E1 as a Molecular Vehicle for Cloning and Amplification of DNA*. Proc. Nat. Acad. Sci., 1974, 71, pp. 3455-3459.

Appendix I-III-B. Wensink, P. C., D. J. Finnegan, J. E. Donelson, and D. S. Hogness, *A System for Mapping DNA Sequences in the Chromosomes of Drosophila Melanogaster*. Cell, 1974, 3, pp. 315-335.

Appendix I-III-C. Tanaka, T., and B. Weisblum, *Construction of a Colicin EI-R Factor Composite Plasmid in Vitro: Means for Amplification of Deoxyribonucleic Acid*. J. Bacteriol., 1975, 121, pp. 354-362.

Appendix I-III-D. Armstrong, K. A., V. Hersfield, and D. R. Helinski, *Gene Cloning and Containment Properties of Plasmid Col E1 and Its Derivatives*, Science, 1977, 196, pp. 172-174.

Appendix I-III-E. Bolivar, F., R. L. Rodriguez, M. C. Betlack, and H. W. Boyer, *Construction and Characterization of New Cloning Vehicles: I. Ampicillin-Resistant Derivative of PMB9*, Gene, 1977, 2, pp. 75-93.

Appendix I-III-F. Cohen, S. N., A. C. W. Chang, H. Boyer, and R. Helling. *Construction of Biologically Functional Bacterial Plasmids in Vitro*. Proc. Natl. Acad. Sci., 1973, 70, pp. 3240-3244.

Appendix I-III-G. Bolivar, F., R. L. Rodriguez, R. J. Greene, M. C. Batlack, H. L. Reyneker, H. W. Boyer, J. H. Cross, and S. Falkow, 1977, *Construction and Characterization of New Cloning Vehicles II. A Multi-Purpose Cloning System*, Gene, 1977, 2, pp. 95-113.

Appendix I-III-H. Thomas, M., J. R. Cameron, and R. W. Davis (1974). *Viable Molecular Hybrids of Bacteriophage Lambda and Eukaryotic DNA*. Proc. Nat. Acad. Sci., 1974, 71, pp. 4579-4583.

Appendix I-III-I. Murray, N. E., and K. Murray, *Manipulation of Restriction Targets in Phage Lambda to Form Receptor Chromosomes for DNA Fragments*. Nature, 1974, 51, pp. 476-481.

Appendix I-III-J. Ramback, A., and P. Tiollais (1974). *Bacteriophage Having EcoRI Endonuclease Sites Only in the Non-Essential Region of the Genome*. Proc. Nat. Acad. Sci., 1974, 71, pp. 3927-3820.

Appendix I-III-K. Blattner, F. R., B. G. Williams, A. E. Bleche, K. Denniston-Thompson, H. E. Faber, L. A. Furlong, D. J. Gunwald, D. O. Kiefer, D. D. Moore, J. W. Shumm, E. L. Sheldon, and O. Smithies, *Charon Phages: Safer Derivatives of Bacteriophage Lambda for DNA Cloning*, Science 1977, 196, pp. 163-169.

Appendix I-III-L. Donoghue, D. J., and P. A. Sharp, *An Improved Lambda Vector: Construction of Model Recombinants Coding for Kanamycin Resistance*, Gene, 1977, 1, pp. 209-227.

Appendix I-III-M. Leder, P., D. Tiemeier and L. Enquist (1977), *EK2 Derivatives of Bacteriophage Lambda Useful in the Cloning of DNA from Higher Organisms: The λ gt WES System*, Science, 1977, 196, pp. 175-177.

Appendix I-III-N. Skalka, A., *Current Status of Coliphage AEK2 Vectors*, Gene, 1978, 3, pp. 29-35.

Appendix I-III-O. Szybalski, W., A. Skalka, S. Gottesman, A. Campbell, and D. Botstein, *Standardized Laboratory Tests for EK2 Certification*, Gene, 1978, 3, pp. 36-38.

APPENDIX J. BIOTECHNOLOGY RESEARCH SUBCOMMITTEE

The National Science and Technology Council's Committee on Fundamental Science determined that a subcommittee should be continued to identify and coordinate Federal research efforts, identify research needs, stimulating international cooperation, and assess national and international policy issues concerning biotechnology sciences. The primary emphasis will be on scientific issues to increase the overall effectiveness and productivity of the Federal investment in biotechnology sciences, especially regarding issues which cut across agency boundaries. This subcommittee is called the Biotechnology Research Subcommittee.

Membership of the Biotechnology Research Subcommittee will include Federal agencies that support biotechnology research. Agencies represented are: U.S. Department of Agriculture, Department of Commerce, Department of Defense, Department of Energy, Department of Health and Human Services, Department of Interior, Department of Justice, Department of State, Department of Veterans Affairs, Agency for International Development, Environmental Protection Agency, National Aeronautics and Space Administration, and National Science Foundation. The Biotechnology Research Subcommittee will function in an advisory capacity to the Committee on Fundamental Science, the Director of the Office of Science and Technology Policy, and the Executive Office of the President. The Biotechnology Research Subcommittee will review the scientific aspects of proposed regulations and guidelines as they are developed.

The primary responsibilities of the Biotechnology Research Subcommittee are to: (i) describe and review current Federal efforts in biotechnology research; (ii) identify and define the priority areas for future Federal biotechnology research, including areas needing greater emphasis, describing the role of each agency in those areas, and delineate where interagency cooperation would enhance progress in the biotechnology sciences, with an emphasis on integrated research efforts, where appropriate; (iii) assess major international efforts in the biotechnology sciences and develop mechanisms for international collaboration. For example, activities of the U.S.-European Community Task Force on Biotechnology have been coordinated through the Biotechnology Research Subcommittee; (iv) identify and review national and international policy issues (such as public education) associated with biotechnology; and (v) provide reviews, analyses, and recommendations to the Chairs of the Committee on Fundamental Science on scientific issues related to regulations and the applications of biotechnology research and biotechnology policies and issues.

In 1990, the Biotechnology Research Subcommittee replaced the Biotechnology Sciences Coordinating Committee. Both the Biotechnology Research Subcommittee and the Biotechnology Sciences Coordinating Committee previously functioned under the Federal Coordinating Council on Science, Engineering, and Technology (FCCSET). While regulatory issues became the primary focus of the Biotechnology Sciences Coordinating Committee, the Biotechnology Research Subcommittee focuses on scientific issues, although it will still provide scientific support for regulatory responsibilities.

APPENDIX K. PHYSICAL CONTAINMENT FOR LARGE SCALE USES OF ORGANISMS CONTAINING RECOMBINANT DNA MOLECULES

Appendix K specifies physical containment guidelines for large-scale (greater than 10 liters of culture) research or production involving viable organisms containing recombinant DNA molecules. It shall apply to large-scale research or production activities as specified in [Section III-D-6, *Experiments Involving More than 10 Liters of Culture*](#). It is important to note that this appendix addresses only the biological hazard associated with organisms containing recombinant DNA. Other hazards accompanying the large-scale cultivation of such organisms (e.g., toxic properties of products; physical, mechanical, and chemical aspects of downstream processing) are not addressed and shall be considered separately, albeit in conjunction with this appendix.

All provisions shall apply to large-scale research or production activities with the following modifications: (i) Appendix K shall supersede [Appendix G, *Physical Containment*](#), when quantities in excess of 10 liters of culture are involved in research or production. [Appendix K-II](#) applies to Good Large Scale Practice; (ii) the institution shall appoint a Biological Safety Officer if it engages in large-scale research or production activities involving viable organisms containing recombinant DNA molecules. The duties of the Biological Safety Officer shall include those specified in [Section IV-B-3, *Biological Safety Officer*](#); (iii) the institution shall establish and maintain a health surveillance program for personnel engaged in large-scale research or production activities involving viable organisms containing recombinant DNA molecules which require Biosafety Level (BL) 3 containment at the laboratory scale. The program shall include: preassignment and periodic physical and medical examinations; collection, maintenance, and analysis of serum specimens for monitoring serologic changes that may result from the employee's work experience; and provisions for the investigation of any serious, unusual, or extended illnesses of employees to determine possible occupational origin.

Appendix K-I. Selection of Physical Containment Levels

The selection of the physical containment level required for recombinant DNA research or production involving more than 10 liters of culture is based on the containment guidelines established in [Section III, *Experiments Covered by the NIH Guidelines*](#). For purposes of large-scale research or production, four physical containment levels are established. The four levels set containment conditions at those appropriate for the degree of hazard to health or the environment posed by the organism, judged by experience with similar organisms unmodified by recombinant DNA techniques and consistent with Good Large Scale Practice. The four biosafety levels of large-scale physical containment are referred to as Good Large Scale Practice, BL1-Large Scale, BL2-Large Scale, and BL3-Large Scale. Good Large Scale Practice is recommended for large-scale research or production involving viable, non-pathogenic, and non-toxigenic recombinant strains derived from host organisms that have an extended history of safe large-scale use. Good Large Scale Practice is recommended for organisms such as those included in [Appendix C, *Exemptions under Section III-F-6*](#), which have built-in environmental limitations that permit optimum growth in the large-scale setting but limited survival without adverse consequences in the environment. BL1-Large Scale is recommended for large-scale research or production of viable organisms containing recombinant DNA molecules that require BL1 containment at the laboratory scale and that do not qualify for Good Large Scale Practice. BL2-Large Scale is recommended for large-scale research or production of viable organisms containing recombinant DNA molecules that require BL2 containment at the laboratory scale. BL3-Large Scale is recommended for large-scale research or production of viable organisms containing recombinant DNA molecules that require BL3 containment at the laboratory scale. No provisions are made for large-scale research or production of viable organisms containing recombinant DNA molecules that require BL4 containment at the laboratory scale. If necessary, these requirements will be established by NIH on an individual basis.

Appendix K-II. Good Large Scale Practice (GLSP)

Appendix K-II-A. Institutional codes of practice shall be formulated and implemented to assure adequate control of health and safety matters.

Appendix K-II-B. Written instructions and training of personnel shall be provided to assure that cultures of viable organisms containing recombinant DNA molecules are handled prudently and that the work place is kept clean and orderly.

Appendix K-II-C. In the interest of good personal hygiene, facilities (e.g., hand washing sink, shower, changing room) and protective clothing (e.g., uniforms, laboratory coats) shall be provided that are appropriate for the risk of exposure to viable organisms containing recombinant DNA molecules. Eating, drinking, smoking, applying cosmetics, and mouth pipetting shall be prohibited in the work area.

Appendix K-II-D. Cultures of viable organisms containing recombinant DNA molecules shall be handled in facilities intended to safeguard health during work with microorganisms that do not require containment.

Appendix K-II-E. Discharges containing viable recombinant organisms shall be handled in accordance with applicable governmental environmental regulations.

Appendix K-II-F. Addition of materials to a system, sample collection, transfer of culture fluids within/between systems, and processing of culture fluids shall be conducted in a manner that maintains employee's exposure to viable organisms containing recombinant DNA molecules at a level that does not adversely affect the health and safety of employees.

Appendix K-II-G. The facility's emergency response plan shall include provisions for handling spills.

Appendix K-III. Biosafety Level 1 (BL1) - Large Scale

Appendix K-III-A. Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Laboratory Director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

Appendix K-III-B. Cultures of viable organisms containing recombinant DNA molecules shall be handled in a closed system (e.g., closed vessel used for the propagation and growth of cultures) or other primary containment equipment (e.g., biological safety cabinet containing a centrifuge used to process culture fluids) which is designed to reduce the potential for escape of viable organisms. Volumes less than 10 liters may be handled outside of a closed system or other primary containment equipment provided all physical containment requirements specified in [Appendix G-II-A, Physical Containment Levels--Biosafety Level 1](#), are met.

Appendix K-III-C. Culture fluids (except as allowed in Appendix K-III-D) shall not be removed from a closed system or other primary containment equipment unless the viable organisms containing recombinant DNA molecules have been inactivated by a validated inactivation procedure. A validated inactivation procedure is one which has been demonstrated to be effective using the organism that will serve as the host for propagating the recombinant DNA molecules. Culture fluids that contain viable organisms or viral vectors intended as final product may be removed from the primary containment equipment by way of closed systems for sample analysis, further processing or final fill.

Appendix K-III-D. Sample collection from a closed system, the addition of materials to a closed system, and the transfer of culture fluids from one closed system to another shall be conducted in a manner which minimizes the release of aerosols or contamination of exposed surfaces.

Appendix K-III-E. Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters which have efficiencies equivalent to high efficiency particulate air/HEPA filters or by other equivalent procedures (e.g., incineration) to minimize the release of viable organisms containing recombinant DNA molecules to the environment.

Appendix K-III-F. A closed system or other primary containment equipment that has contained viable organisms containing recombinant DNA molecules shall not be opened for maintenance or other purposes unless it has been sterilized by a validated sterilization procedure except when the culture fluids contain viable organisms or vectors intended as final product as described in [Appendix K-III-C](#) above. A validated sterilization procedure is one which has been demonstrated to be effective using the organism that will serve as the host for propagating the recombinant DNA molecules.

Appendix K-III-G. Emergency plans required by [Sections IV-B-2-b-\(6\)](#), *Institutional Biosafety Committee*, and [IV-B-3-c-\(3\)](#), *Biological Safety Officer*, shall include methods and procedures for handling large losses of culture on an emergency basis.

Appendix K-IV. Biosafety Level 2 (BL2) - Large Scale

Appendix K-IV-A. Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities (if applicable). Reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

Appendix K-IV-B. Cultures of viable organisms containing recombinant DNA molecules shall be handled in a closed system (e.g., closed vessel used for the propagation and growth of cultures) or other primary containment equipment (e.g., Class III biological safety cabinet containing a centrifuge used to process culture fluids) which is designed to prevent the escape of viable organisms. Volumes less than 10 liters may be handled outside of a closed system or other primary containment equipment provided all physical containment requirements specified in [Appendix G-II-B, Physical Containment Levels--Biosafety Level 2](#), are met.

Appendix K-IV-C. Culture fluids (except as allowed in Appendix K-IV-D) shall not be removed from a closed system or other primary containment equipment unless the viable organisms containing recombinant DNA molecules have been inactivated by a validated inactivation procedure. A validated inactivation procedure is one which has been demonstrated to be effective using the organism that will serve as the host for propagating the recombinant DNA molecules. Culture fluids that contain viable organisms or viral vectors intended as final product may be removed from the primary containment equipment by way of closed systems for sample analysis, further processing or final fill.

Appendix K-IV-D. Sample collection from a closed system, the addition of materials to a closed system, and the transfer of cultures fluids from one closed system to another shall be conducted in a manner which prevents the release of aerosols or contamination of exposed surfaces.

Appendix K-IV-E. Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters which have efficiencies equivalent to high efficiency particulate air/HEPA filters or by other equivalent procedures (e.g., incineration) to prevent the release of viable organisms containing recombinant DNA molecules to the environment.

Appendix K-IV-F. A closed system or other primary containment equipment that has contained viable organisms containing recombinant DNA molecules shall not be opened for maintenance or other purposes unless it has been sterilized by a validated sterilization procedure except when the culture fluids contain viable organisms or vectors intended as final product as described in [Appendix K-IV-C](#) above. A validated sterilization procedure is one which has been demonstrated to be effective using the organisms that will serve as the host for propagating the recombinant DNA molecules.

Appendix K-IV-G. Rotating seals and other mechanical devices directly associated with a closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be designed to prevent leakage or shall be fully enclosed in ventilated housings that are exhausted through filters which have efficiencies equivalent to high efficiency particulate air/HEPA filters or through other equivalent treatment devices.

Appendix K-IV-H. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules and other primary containment equipment used to contain operations involving viable organisms containing sensing devices that monitor the integrity of containment during operations.

Appendix K-IV-I. A closed system used for the propagation and growth of viable organisms containing the recombinant DNA molecules shall be tested for integrity of the containment features using the organism that will serve as the host for propagating recombinant DNA molecules. Testing shall be accomplished prior to the introduction of viable organisms containing recombinant DNA molecules and following modification or replacement of essential containment features. Procedures and methods used in the testing shall be appropriate for the equipment design and for recovery and demonstration of the test organism. Records of tests and results shall be maintained on file.

Appendix K-IV-J. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be permanently identified. This identification shall be used in all records reflecting testing, operation, and maintenance and in all documentation relating to use of this equipment for research or production activities involving viable organisms containing recombinant DNA molecules.

Appendix K-IV-K. The universal biosafety sign shall be posted on each closed system and primary containment equipment when used to contain viable organisms containing recombinant DNA molecules.

Appendix K-IV-L. Emergency plans required by [Sections IV-B-2-b-\(6\)](#), *Institutional Biosafety Committee*, and [IV-B-3-c-\(3\)](#), *Biological Safety Officer*, shall include methods and procedures for handling large losses of culture on an emergency basis.

Appendix K-V. Biosafety Level 3 (BL3) - Large Scale

Appendix K-V-A. Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities (if applicable). Reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

Appendix K-V-B. Cultures of viable organisms containing recombinant DNA molecules shall be handled in a closed system (e.g., closed vessels used for the propagation and growth of cultures) or other primary containment equipment (e.g., Class III biological safety cabinet containing a centrifuge used to process culture fluids) which is designed to prevent the escape of viable organisms. Volumes less than 10 liters may be handled outside of a closed system provided all physical containment requirements specified in [Appendix G-II-C](#), *Physical Containment Levels--Biosafety Level 3*, are met.

Appendix K-V-C. Culture fluids (except as allowed in Appendix K-V-D) shall not be removed from a closed system or other primary containment equipment unless the viable organisms containing recombinant DNA molecules have been inactivated by a validated inactivation procedure. A validated inactivation procedure is one which has been demonstrated to be effective using the organisms that will serve as the host for propagating the recombinant DNA molecules. Culture fluids that contain viable organisms or viral vectors intended as final product may be removed from the primary containment equipment by way of closed systems for sample analysis, further processing or final fill.

Appendix K-V-D. Sample collection from a closed system, the addition of materials to a closed system, and the transfer of culture fluids from one closed system to another shall be conducted in a manner which prevents the release of aerosols or contamination of exposed surfaces.

Appendix K-V-E. Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters which have efficiencies equivalent to high efficiency particulate air/HEPA filters or by other equivalent procedures (e.g., incineration) to prevent the release of viable organisms containing recombinant DNA molecules to the environment.

Appendix K-V-F. A closed system or other primary containment equipment that has contained viable organisms containing recombinant DNA molecules shall not be opened for maintenance or other purposes unless it has been sterilized by a validated sterilization procedure except when the culture fluids contain viable organisms or vectors intended as final product as described in [Appendix K-V-C](#) above. A validated sterilization procedure is one which has been demonstrated to be effective using the organisms that will serve as the host for propagating the recombinant DNA molecules.

Appendix K-V-G. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be operated so that the space above the culture level will be maintained at a pressure as low as possible, consistent with equipment design, in order to maintain the integrity of containment features.

Appendix K-V-H. Rotating seals and other mechanical devices directly associated with a closed system used to contain viable organisms containing recombinant DNA molecules shall be designed to prevent leakage or shall be fully enclosed in ventilated housings that are exhausted through filters which have efficiencies equivalent to high efficiency particulate air/HEPA filters or through other equivalent treatment devices.

Appendix K-V-I. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules and other primary containment equipment used to contain operations involving viable organisms containing recombinant DNA molecules shall include monitoring or sensing devices that monitor the integrity of containment during operations.

Appendix K-V-J. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be tested for integrity of the containment features using the organisms that will serve as the host for propagating the recombinant DNA molecules. Testing shall be accomplished prior to the introduction of viable organisms containing recombinant DNA molecules and following modification or replacement of essential containment features. Procedures and methods used in the testing shall be appropriate for the equipment design and for recovery and demonstration of the test organism. Records of tests and results shall be maintained on file.

Appendix K-V-K. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be permanently identified. This identification shall be used in all records reflecting testing, operation, maintenance, and use of this equipment for research production activities involving viable organisms containing recombinant DNA molecules.

Appendix K-V-L. The universal biosafety sign shall be posted on each closed system and primary containment equipment when used to contain viable organisms containing recombinant DNA molecules.

Appendix K-V-M. Emergency plans required by [Sections IV-B-2-b\(6\)](#), *Institutional Biosafety Committee*, and [IV-B-3-c\(3\)](#), *Biological Safety Officer*, shall include methods and procedures for handling large losses of culture on an emergency basis.

Appendix K-V-N. Closed systems and other primary containment equipment used in handling cultures of viable organisms containing recombinant DNA molecules shall be located within a controlled area which meets the following requirements:

Appendix K-V-N-1. The controlled area shall have a separate entry area. The entry area shall be a double-doored space such as an air lock, anteroom, or change room that separates the controlled area from the balance of the facility.

Appendix K-V-N-2. The surfaces of walls, ceilings, and floors in the controlled area shall be such as to permit ready cleaning and decontamination.

Appendix K-V-N-3. Penetrations into the controlled area shall be sealed to permit liquid or vapor phase space decontamination.

Appendix K-V-N-4. All utilities and service or process piping and wiring entering the controlled area shall be protected against contamination.

Appendix K-V-N-5. Hand washing facilities equipped with foot, elbow, or automatically operated valves shall be located at each major work area and near each primary exit.

Appendix K-V-N-6. A shower facility shall be provided. This facility shall be located in close proximity to the controlled area.

Appendix K-V-N-7. The controlled area shall be designed to preclude release of culture fluids outside the controlled area in the event of an accidental spill or release from the closed systems or other primary containment equipment.

Appendix K-V-N-8. The controlled area shall have a ventilation system that is capable of controlling air movement. The movement of air shall be from areas of lower contamination potential to areas of higher contamination potential. If the ventilation system provides positive pressure supply air, the system shall operate in a manner that prevents the reversal of the direction of air movement or shall be equipped with an alarm that would be actuated in the event that reversal in the direction of air movement were to occur. The exhaust air from the controlled area shall not be recirculated to other areas of the facility. The exhaust air from the controlled area may not be discharged to the outdoors without being high efficiency particulate air/HEPA filtered, subjected to thermal oxidation, or otherwise treated to prevent the release of viable organisms.

Appendix K-V-O. The following personnel and operational practices shall be required:

Appendix K-V-O-1. Personnel entry into the controlled area shall be through the entry area specified in Appendix K-V-N-1.

Appendix K-V-O-2. Persons entering the controlled area shall exchange or cover their personal clothing with work garments such as jump suits, laboratory coats, pants and shirts, head cover, and shoes or shoe covers. On exit from the controlled area the work clothing may be stored in a locker separate from that used for personal clothing or discarded for laundering. Clothing shall be decontaminated before laundering.

Appendix K-V-O-3. Entry into the controlled area during periods when work is in progress shall be restricted to those persons required to meet program or support needs. Prior to entry, all persons shall be informed of the operating practices, emergency procedures, and the nature of the work conducted.

Appendix K-V-O-4. Persons under 18 years of age shall not be permitted to enter the controlled area.

Appendix K-V-O-5. The universal biosafety sign shall be posted on entry doors to the controlled area and all internal doors when any work involving the organism is in progress. This includes periods when decontamination procedures are in progress. The sign posted on the entry doors to the controlled area shall include a statement of agents in use and personnel authorized to enter the controlled area.

Appendix K-V-O-6. The controlled area shall be kept neat and clean.

Appendix K-V-O-7. Eating, drinking, smoking, and storage of food are prohibited in the controlled area.

Appendix K-V-O-8. Animals and plants shall be excluded from the controlled area.

Appendix K-V-O-9. An effective insect and rodent control program shall be maintained.

Appendix K-V-O-10. Access doors to the controlled area shall be kept closed, except as necessary for access, while work is in progress. Service doors leading directly outdoors shall be sealed and locked while work is in progress.

Appendix K-V-O-11. Persons shall wash their hands when exiting the controlled area.

Appendix K-V-O-12. Persons working in the controlled area shall be trained in emergency procedures.

Appendix K-V-O-13. Equipment and materials required for the management of accidents involving viable organisms containing recombinant DNA molecules shall be available in the controlled area.

Appendix K-V-O-14. The controlled area shall be decontaminated in accordance with established procedures following spills or other accidental release of viable organisms containing recombinant DNA molecules.

Appendix K - Table 1. Comparison of Good Large Scale Practice (GLSP) and Biosafety Level (BL) - Large Scale (LS) Practice (See [Appendix K-VI-A](#), *Footnotes Of Appendix K*)

CRITERION [See Appendix K-VI-B , <i>Footnotes of Appendix K</i>]		GLSP	BL1-LS	BL2-LS	BL3-LS
1.	Formulate and implement institutional codes of practice for safety of personnel and adequate control of hygiene and safety measures.	K-II-A	G-I		
2.	Provide adequate written instructions and training of personnel to keep work place clean and tidy and to keep exposure to biological, chemical or physical agents at a level that does not adversely affect health and safety of employees.	K-II-B	G-I		
3.	Provide changing and hand washing facilities as well as protective clothing, appropriate to the risk, to be worn during work.	K-II-C	G-II-A-1-h	G-II-B-2-f	G-II-C-2-i
4.	Prohibit eating, drinking, smoking, mouth pipetting, and applying cosmetics in the work place.	K-II-C	G-II-A-1-d G-II-A-1-e	G-II-B-1-d G-II-B-1-e	G-II-C-1-c G-II-C-1-d
5.	Internal accident reporting.	K-II-G	K-III-A	K-IV-A	K-V-A
6.	Medical surveillance.	NR	NR		
7.	Viable organisms should be handled in a system that physically separates the process from the external environment (closed system or other primary containment).	NR	K-III-B	K-IV-B	K-V-B
8.	Culture fluids not removed from a system until organisms are inactivated.	NR	K-III-C	K-IV-C	K-V-C
9.	Inactivation of waste solutions and materials with respect to their biohazard potential.	K-II-E	K-III-C	K-IV-C	K-V-C
10.	Control of aerosols by engineering or procedural controls to prevent or minimize release of organisms during sampling from a system, addition of materials to a system, transfer of cultivated cells, and removal of material, products, and effluent from a system.	Minimize <i>Procedure</i> K-II-F	Minimize <i>Engineer</i> K-III-B K-III-D	Prevent <i>Engineer</i> K-IV-B K-IV-D	Prevent <i>Engineer</i> K-V-B K-V-D
11.	Treatment of exhaust gases from a closed system to minimize or prevent release of viable organisms.	NR	Minimize K-III-E	Prevent K-IV-E	Prevent K-V-E
12.	Closed system that has contained viable organisms not to be opened until sterilized by a validated procedure.	NR	K-III-F	K-IV-F	K-V-F
13.	Closed system to be maintained at as a low pressure as possible to maintain integrity of containment features.	NR	NR	NR	K-V-G
14.	Rotating seals and other penetrations into closed system designed to prevent or minimize leakage.	NR	NR	Prevent K-IV-G	Prevent K-V-H
15.	Closed system shall incorporate monitoring or sensing devices to monitor the integrity of containment.	NR	NR	K-IV-H	K-V-I
16.	Validated integrity testing of closed containment system.	NR	NR	K-IV-I	K-V-J
17.	Closed system to be permanently identified for record keeping purposes.	NR	NR	K-IV-J	K-V-K
18.	Universal biosafety sign to be posted on each closed system.	NR	NR	K-IV-K	K-V-L
19.	Emergency plans required for handling large losses of cultures.	K-II-G	K-III-G	K-IV-L	K-V-M
20.	Access to the work place.	NR	G-II-A-1-a	G-II-B-1-a	K-V-N
21.	Requirements for controlled access area.	NR	NR	NR	K-V-N&O

NR = not required

Appendix K-VI. Footnotes of Appendix K

Appendix K-VI-A. This table is derived from the text in Appendices G (*Physical Containment*) and K and is not to be used in lieu of Appendices G and K.

Appendix K-VI-B. The criteria in this grid address only the biological hazards associated with organisms containing recombinant DNA. Other hazards accompanying the large-scale cultivation of such organisms (e.g., toxic properties of products; physical, mechanical, and chemical aspects of downstream processing) are not addressed and shall be considered separately, albeit in conjunction with this grid.

Appendix K-VII. Definitions to Accompany Containment Grid and Appendix K

Appendix K-VII-A. Accidental Release. An accidental release is the unintentional discharge of a microbiological agent (i.e., microorganism or virus) or eukaryotic cell due to a failure in the containment system.

Appendix K-VII-B. Biological Barrier. A biological barrier is an impediment (naturally occurring or introduced) to the infectivity and/or survival of a microbiological agent or eukaryotic cell once it has been released into the environment.

Appendix K-VII-C. Closed System. A closed system is one in which by its design and proper operation, prevents release of a microbiological agent or eukaryotic cell contained therein.

Appendix K-VII-D. Containment. Containment is the confinement of a microbiological agent or eukaryotic cell that is being cultured, stored, manipulated, transported, or destroyed in order to prevent or limit its contact with people and/or the environment. Methods used to achieve this include: physical and biological barriers and inactivation using physical or chemical means.

Appendix K-VII-E. De minimis Release. *De minimis* release is the release of: (i) viable microbiological agents or eukaryotic cells that does not result in the establishment of disease in healthy people, plants, or animals; or (ii) in uncontrolled proliferation of any microbiological agents or eukaryotic cells.

Appendix K-VII-F. Disinfection. Disinfection is a process by which viable microbiological agents or eukaryotic cells are reduced to a level unlikely to produce disease in healthy people, plants, or animals.

Appendix K-VII-G. Good Large Scale Practice Organism. For an organism to qualify for Good Large Scale Practice consideration, it must meet the following criteria [Reference: Organization for Economic Cooperation and Development, *Recombinant DNA Safety Considerations*, 1987, p. 34-35]: (i) the host organism should be non-pathogenic, should not contain adventitious agents and should have an extended history of safe large-scale use or have built-in environmental limitations that permit optimum growth in the large-scale setting but limited survival without adverse consequences in the environment; (ii) the recombinant DNA-engineered organism should be non-pathogenic, should be as safe in the large-scale setting as the host organism, and without adverse consequences in the environment; and (iii) the vector/insert should be well characterized and free from known harmful sequences; should be limited in size as much as possible to the DNA required to perform the intended function; should not increase the stability of the construct in the environment unless that is a requirement of the intended function; should be poorly mobilizable; and should not transfer any resistance markers to microorganisms unknown to acquire them naturally if such acquisition could compromise the use of a drug to control disease agents in human or veterinary medicine or agriculture.

Appendix K-VII-H. Inactivation. Inactivation is any process that destroys the ability of a specific microbiological agent or eukaryotic cell to self-replicate.

Appendix K-VII-I. Incidental Release. An incidental release is the discharge of a microbiological agent or eukaryotic cell from a containment system that is expected when the system is appropriately designed and properly operated and maintained.

Appendix K-VII-J. Minimization. Minimization is the design and operation of containment systems in order that any incidental release is a *de minimis* release.

Appendix K-VII-K. Pathogen. A pathogen is any microbiological agent or eukaryotic cell containing sufficient genetic information, which upon expression of such information, is capable of producing disease in healthy people, plants, or animals.

Appendix K-VII-L. Physical Barrier. A physical barrier is considered any equipment, facilities, or devices (e.g., fermentors, factories, filters, thermal oxidizers) which are designed to achieve containment.

Appendix K-VII-M. Release. Release is the discharge of a microbiological agent or eukaryotic cell from a containment system. Discharges can be incidental or accidental. Incidental releases are *de minimis* in nature; accidental releases may be *de minimis* in nature.

APPENDIX L. GENE THERAPY POLICY CONFERENCES (GTPCS)

In order to enhance the depth and value of public discussion relevant to scientific, safety, social, and ethical implications of gene therapy research, the NIH Director will convene GTPCs at regular intervals. As appropriate, the NIH Director may convene a GTPC in conjunction with a RAC meeting. GTPCs will be administered by NIH/OBA. Conference participation will not involve a standing committee membership but rather will offer the unique advantage of assembling numerous participants who possess significant scientific, ethical, and legal expertise and/or interest that is directly applicable to a specific gene therapy research issue. At least one member of RAC will serve as Co-chair of each GTPC and report the findings of each GTPC to RAC at its next scheduled meeting. The RAC representative for each GTPC will be chosen based on the participant's area of expertise relative to the specific gene therapy research issue to be discussed. All RAC members will be invited to attend GTPCs. GTPCs will have representation from other Federal agencies, including [FDA](#) and [OHRP](#). GTPCs will focus on broad overarching policy and scientific issues related to gene therapy research. Proposals for GTPC topics may be submitted by members of RAC, representatives of academia, industry, patient and consumer advocacy organizations, other Federal agencies, professional scientific societies, and the general public. GTPC topics will not be limited to discussion of human applications of gene therapy research, i.e., they may include basic research on the use of novel gene delivery vehicles, or novel applications of human gene transfer. The RAC, with the Director's approval, will have the primary responsibility for planning GTPC agendas. GTPC findings will be transmitted to the NIH Director and will be made publicly available. The NIH Director anticipates that this public policy forum will serve as a model for interagency communication and collaboration, concentrated expert discussion of novel scientific issues and their potential societal implications, and enhanced opportunity for public discussion of specific issues and potential impact of such applications on human health and the environment.

APPENDIX M. POINTS TO CONSIDER IN THE DESIGN AND SUBMISSION OF PROTOCOLS FOR THE TRANSFER OF RECOMBINANT DNA MOLECULES INTO ONE OR MORE HUMAN RESEARCH PARTICIPANTS (POINTS TO CONSIDER)

Appendix M applies to research conducted at or sponsored by an institution that receives any support for recombinant DNA research from NIH. Researchers not covered by the *NIH Guidelines* are encouraged to use Appendix M (see [Section I-C](#), *General Applicability*).

The acceptability of human somatic cell gene transfer has been addressed in several public documents as well as in numerous academic studies. In November 1982, the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research published a report, *Splicing Life*, which resulted from a two-year process of public deliberation and hearings. Upon release of that report, a U.S. House of Representatives subcommittee held three days of public hearings with witnesses from a wide range of fields from the biomedical and social sciences to theology, philosophy, and law. In December 1984, the Office of Technology Assessment released a background paper, *Human Gene Therapy*, which concluded that civic, religious, scientific, and medical groups have all accepted, in principle, the appropriateness of gene transfer of somatic cells in humans for specific genetic diseases. Somatic cell gene transfer is seen as an extension of present methods that might be preferable to other technologies. In light of this public support, RAC is prepared to consider proposals for somatic cell gene transfer.

RAC will not at present entertain proposals for germ line alterations but will consider proposals involving somatic cell gene transfer. The purpose of somatic cell gene transfer is to treat an individual patient, e.g., by inserting a properly functioning gene into the subject's somatic cells. Germ line alteration involves a specific attempt to introduce genetic changes into the germ (reproductive) cells of an individual, with the aim of changing the set of genes passed on to the individual's offspring.

The RAC continues to explore the issues raised by the potential of *in utero* gene transfer clinical research. However, the RAC concludes that, at present, it is premature to undertake any *in utero* gene transfer clinical trial. Significant additional preclinical and clinical studies addressing vector transduction efficacy, biodistribution, and toxicity are required before a human *in utero* gene transfer protocol can proceed. In addition, a more thorough understanding of the development of human organ systems, such as the immune and nervous systems, is needed to better define the potential efficacy and risks of human *in utero* gene transfer. Prerequisites for considering any specific human *in utero* gene transfer procedure include an understanding of the pathophysiology of the candidate disease and a demonstrable advantage to the *in utero* approach. Once the above criteria are met, the RAC would be willing to consider well rationalized human *in utero* gene transfer clinical trials.

Research proposals involving the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into human subjects (human gene transfer) will be considered through a review process involving both NIH/OBA and RAC. Investigators shall submit their relevant information on the proposed human gene transfer experiments to NIH/OBA. Submission of human gene transfer protocols to NIH will be in the format described in [Appendix M-I-A, Submission Requirements for Protocol Submission](#). Submission to NIH shall be for registration purposes and will ensure continued public access to relevant human gene transfer information conducted in compliance with the *NIH Guidelines*. Investigational New Drug (IND) applications should be submitted to [FDA](#) in the format described in 21 CFR, Chapter I, Subchapter D, Part 312, Subpart B, Section 23, *IND Content and Format*.

Institutional Biosafety Committee approval must be obtained from each institution at which recombinant DNA material will be administered to human subjects (as opposed to each institution involved in the production of vectors for human application and each institution at which there is *ex vivo* transduction of recombinant DNA material into target cells for human application).

Factors that may contribute to public discussion of a human gene transfer experiment by RAC include: (i) new vectors/new gene delivery systems, (ii) new diseases, (iii) unique applications of gene transfer, and (iv) other issues considered to require further public discussion. Among the experiments that may be considered exempt from RAC discussion are those determined not to represent possible risk to human health or the environment. Full, public RAC review and discussion of a human gene transfer experiment may be (1) initiated by the NIH Director; or (2) initiated by the NIH OBA Director following a recommendation to NIH OBA by: (a) three or more RAC members, or (b) a Federal agency other than NIH. An individual human gene transfer experiment that is recommended for full RAC review should represent novel characteristics deserving of public discussion. If it is determined that an experiment will undergo full RAC discussion, NIH/OBA will immediately notify the Principal Investigator. RAC members may forward individual requests for additional information relevant to a specific protocol through NIH/OBA to the Principal Investigator. In making a determination whether an experiment is novel, and thus deserving of full RAC discussion, reviewers will examine the scientific rationale, scientific context (relative to other proposals reviewed by RAC), whether the preliminary *in vitro* and *in vivo* safety data were obtained in appropriate models and are sufficient, and whether questions related to relevant social and ethical issues have been resolved. RAC recommendations on a specific human gene transfer experiment shall be forwarded to the NIH Director, the Principal Investigator, the sponsoring institution, and other DHHS components, as appropriate. Relevant documentation will be included in the material for the RAC meeting at which the experiment is scheduled to be discussed. RAC meetings will be open to the public except where trade secrets and proprietary information are reviewed (see [Section IV-D-5, Protection of Proprietary Data – Voluntary Compliance](#)). RAC prefers that information provided in response to Appendix M contain no proprietary data or trade secrets, enabling all aspects of the review to be open to the public.

Note: Any application submitted to NIH/OBA shall not be designated as 'confidential' in its entirety. In the event that a sponsor determines that specific responses to one or more of the items described in Appendix M should be considered as proprietary or trade secret, each item should be clearly identified as such. The cover letter (attached to the submitted material) shall: (1) clearly indicate that select portions of the application contain information considered as proprietary or trade secret, (2) a brief explanation as to the reason that each of these items is determined proprietary or trade secret.

Public discussion of human gene transfer experiments (and access to relevant information) shall serve to inform the public about the technical aspects of the proposals, meaning and significance of the research, and significant safety, social, and ethical implications of the research. RAC discussion is intended to ensure safe and ethical conduct of gene transfer experiments and facilitate public understanding of this novel area of biomedical research.

In its evaluation of human gene transfer proposals, RAC will consider whether the design of such experiments offers adequate assurance that their consequences will not go beyond their purpose, which is the same as the traditional purpose of clinical investigation, namely, to protect the health and well being of human subjects being treated while at the same time gathering generalizable knowledge. Two possible undesirable consequences of the transfer of recombinant DNA would be unintentional: (i) vertical transmission of genetic changes from an individual to his/her offspring, or (ii) horizontal transmission of viral infection to other persons with whom the individual comes in contact. Accordingly, Appendices M-I through M-V request information that will enable RAC and NIH/OBA to assess the possibility that the proposed experiment(s) will inadvertently affect reproductive cells or lead to infection of other people (e.g., medical personnel or relatives).

Appendix M will be considered for revisions as experience in evaluating proposals accumulates and as new scientific developments occur. This review will be carried out periodically as needed.

Appendix M-I. Requirements for Protocol Submission, Review, and Reporting – Human Gene Transfer Experiments

Appendix M-I-A. Requirements for Protocol Submission

The following documentation must be submitted (see exemption in [Appendix M-VI-A, Footnotes of Appendix M](#)) in printed or electronic form to the: Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax), E-mail: rosenthg@od.nih.gov. NIH OBA will confirm receipt within three working days after receiving the submission. Investigators should contact OBA if they do not receive this confirmation.

1. A cover letter on institutional letterhead, signed by the Principal Investigator(s), that: (1) acknowledges that the documentation submitted to NIH OBA complies with the requirements set forth in Appendix M-I-A, *Requirements for Protocol Submission*; (2) identifies the Institutional Biosafety Committee (IBC) and Institutional Review Board (IRB) at the proposed clinical trial site(s) responsible for local review and approval of the protocol; and (3) acknowledges that no research participant will be enrolled (see definition of enrollment in [Section I-E-7](#)) until the RAC review process has been completed (see [Appendix M-I-B, RAC Review Requirements](#)); IBC approval (from the clinical trial site) has been obtained; IRB approval has been obtained; and all applicable regulatory authorizations have been obtained.
2. The scientific abstract.
3. The non-technical abstract.
4. The proposed clinical protocol, including tables, figures, and relevant manuscripts.
5. Responses to Appendices M-II through M-V, *Description of the Proposal, Informed Consent, Privacy and Confidentiality, and Special Issues*. Responses to Appendices M-II through M-V may be provided either as an appendix to the clinical protocol or incorporated in the clinical protocol. If responses to [Appendices M-II through M-V](#) are incorporated in the clinical protocol, each response must refer to the appropriate Appendix M-II through M-V.

6. The proposed informed consent document (see [Appendix M-III, Informed Consent](#)).
7. Curriculum vitae of the principal investigator(s) (no more than two pages in biographical sketch format).

Note: A human gene transfer experiment submitted to NIH OBA should not contain confidential commercial information or trade secrets, enabling all aspects of the review to be open to the public.

Appendix M-I-B. RAC Review Requirements

Appendix M-I-B-1. Initial RAC Review

The initial RAC review process shall include a determination as to whether the human gene transfer experiment presents characteristics that warrant public RAC review and discussion. During the RAC's initial review, individual committee members may request additional information relevant to the protocol. NIH OBA will immediately notify the Principal Investigator(s) of RAC requests for additional information. In making a determination whether an experiment presents characteristics warranting public RAC review and discussion, reviewers will examine the scientific rationale, scientific content, whether the preliminary *in vitro* and *in vivo* safety data were obtained in appropriate models and are sufficient, and whether questions related to relevant social and ethical issues have been resolved. Other factors that may warrant public review and discussion of a human gene transfer experiment by the RAC include: (1) a new vector/new gene delivery system; (2) a new clinical application; (3) a unique application of gene transfer; and/or (4) other issues considered to require further public discussion.

Initial RAC review shall be completed within 15 working days of receipt of a complete submission (see [Appendix M-I-A, Requirements for Protocol Submission](#)). At the end of the 15-day review period, NIH OBA will notify the Principal Investigator(s) in writing about the results of the RAC's initial review. Two outcomes are possible: (1) the experiment does not present characteristics that warrant further review and discussion and is therefore exempt from public RAC review and discussion; or (2) the experiment presents characteristics that warrant public RAC review and discussion. Completion of the RAC review process is defined as: (1) receipt by the Principal Investigator(s) of a letter from NIH OBA indicating that the submission does not present characteristics that warrant public RAC review and discussion; or (2) receipt by the Principal Investigator(s) of a letter from NIH OBA after public RAC review that summarizes the committee's key comments and recommendations (if any).

If a human gene transfer protocol is submitted less than eight weeks before a scheduled RAC meeting and is subsequently recommended for public RAC review and discussion, the review of the protocol by the RAC will be deferred until the next scheduled RAC meeting. This eight-week period is needed to ensure adequate time for public notice and comment and thorough review by the committee members.

No research participant shall be enrolled (see definition of enrollment in [Section I-E-7](#)) in the human gene transfer experiment until: (1) the RAC review process has been completed; (2) Institutional Biosafety Committee (IBC) approval (from the clinical trial site) has been obtained; (3) Institutional Review Board (IRB) approval has been obtained; and (4) all applicable regulatory authorization(s) have been obtained.

For a clinical trial site that is added after the RAC review process, no research participant shall be enrolled (see definition of enrollment in [Section I-E-7](#)) at the clinical trial site until the following documentation has been submitted to NIH OBA: (1) IBC approval (from the clinical trial site); (2) IRB approval; (3) IRB-approved informed consent document; (4) curriculum vitae of the principal investigator(s) (no more than two pages in biographical sketch format); and (5) NIH grant numbers(s) if applicable.

Appendix M-I-B-2. Public RAC Review and Discussion

Public RAC review and discussion of a human gene transfer experiment may be: (1) initiated by the NIH Director; or (2) initiated by the NIH OBA Director following a recommendation to NIH OBA by: (a) three or more RAC members; or (b) a Federal agency other than NIH. In making a determination whether an experiment presents characteristics warranting public RAC review and discussion, reviewers will examine the scientific rationale, scientific content, whether the preliminary *in vitro* and *in vivo* safety data were obtained in appropriate models and are sufficient, and whether questions related to relevant social and ethical issues have been

resolved. Other factors that may warrant public review and discussion of a human gene transfer experiment by the RAC include: (1) a new vector/new gene delivery system; (2) a new clinical application; (3) a unique application of gene transfer; and/or (4) other issues considered to require further public discussion.

After a human gene transfer experiment is reviewed by the full RAC at a regularly scheduled meeting, NIH OBA will send a letter summarizing the RAC key comments and recommendations (if any) regarding the protocol to the NIH Director, the Principal Investigator, the sponsoring institution, and other DHHS components, as appropriate. Completion of RAC review is defined as receipt by the Principal Investigator(s) of a letter from NIH OBA summarizing the committee's findings. Unless NIH OBA determines that there are exceptional circumstances, the RAC summary letter will be sent to the Principal Investigator(s) within 10 working days after the completion of the RAC meeting at which the experiment was reviewed.

RAC meetings will be open to the public except where trade secrets or confidential commercial information are reviewed. To enable all aspects of the protocol review process to be open to the public, information provided in response to Appendix M should not contain trade secrets or confidential commercial information. No application submitted to NIH OBA shall be designated as 'confidential' in its entirety. In the event that an investigator determines that specific responses to one or more of the items described in Appendix M should be considered as confidential commercial information or a trade secret, each item must be clearly identified as such. The cover letter (attached to the submitted material) shall: (1) clearly designate the information that is considered as confidential commercial information or a trade secret; and (2) explain and justify each designation.

Appendix M-I-C. Reporting Requirements

Appendix M-I-C-1. Initiation of the Clinical Investigation

No later than 20 working days after enrollment (see definition of enrollment in [Section I-E-7](#)) of the first research participant in a human gene transfer experiment, the Principal Investigator(s) shall submit the following documentation to NIH OBA: (1) a copy of the informed consent document approved by the Institutional Review Board (IRB); (2) a copy of the protocol approved by the Institutional Biosafety Committee (IBC) and IRB; (3) a copy of the final IBC approval from the clinical trial site; (4) a copy of the final IRB approval; (5) a brief written report that includes the following information: (a) how the investigator(s) responded to each of the RAC's recommendations on the protocol (if applicable); and (b) any modifications to the protocol as required by [FDA](#); (6) applicable NIH grant number(s); (7) the [FDA Investigational New Drug Application \(IND\)](#) number; and (8) the date of the initiation of the trial. The purpose of requesting the FDA IND number is for facilitating interagency collaboration in the Federal oversight of human gene transfer research.

Appendix M-I-C-2. Additional Clinical Trial Sites

No research participant shall be enrolled (see definition of enrollment in [Section I-E-7](#)) at a clinical trial site until the following documentation has been submitted to NIH OBA: (1) Institutional Biosafety Committee approval (from the clinical trial site); (2) Institutional Review Board approval; (3) Institutional Review Board-approved informed consent document; (4) curriculum vitae of the principal investigator(s) (no more than two pages in biographical sketch format); and (5) NIH grant number(s) if applicable.

Appendix M-I-C-3. Annual Reports

Within 60 days after the one-year anniversary of the date on which the investigational new drug (IND) application went into effect, and after each subsequent anniversary until the trial is completed, the Principal Investigator (or delegate) shall submit the information set forth in (a), (b), and (c). When multiple studies are conducted under the single IND, the Principal Investigator (or delegate) may choose to submit a single annual report covering all studies, provided that each study is identified by its OBA protocol number.

(a) Clinical Trial Information. A brief summary of the status of each trial in progress and each trial completed during the previous year. The summary is required to include the following information for each trial: (1) the title and purpose of the trial; (2) clinical site; (3) the Principal Investigator; (4) clinical protocol identifiers, including the NIH OBA protocol number, NIH grant number(s) (if applicable), and the FDA IND application number; (5) participant population (such as disease indication and general age group, e.g., adult or pediatric); (6) the total number of participants planned for inclusion in the trial; the number entered into the trial to date; the number

whose participation in the trial was completed; and the number who dropped out of the trial with a brief description of the reasons; (7) the status of the trial, e.g., open to accrual of subjects, closed but data collection ongoing, or fully completed, and (8) if the trial has been completed, a brief description of any study results.

(b) Progress Report and Data Analysis. Information obtained during the previous year's clinical and non-clinical investigations, including: (1) a narrative or tabular summary showing the most frequent and most serious adverse experiences by body system; (2) a summary of all serious adverse events submitted during the past year; (3) a summary of serious adverse events that were expected or considered to have causes not associated with the use of the gene transfer product such as disease progression or concurrent medications; (4) if any deaths have occurred, the number of participants who died during participation in the investigation and causes of death; and (5) a brief description of any information obtained that is pertinent to an understanding of the gene transfer product's actions, including, for example, information about dose-response, information from controlled trials, and information about bioavailability.

(c) A copy of the updated clinical protocol including a technical and non-technical abstract.

Appendix M-I-C-4. Safety Reporting

Principal Investigators must submit, in accordance with this section, Appendix M-I-C-4-a and [Appendix M-I-C-4-b](#), a written report on: (1) any serious adverse event that is both unexpected and associated with the use of the gene transfer product (i.e., there is reasonable possibility that the event may have been caused by the use of the product; investigators should not await definitive proof of association before reporting such events); and (2) any finding from tests in laboratory animals that suggests a significant risk for human research participants including reports of mutagenicity, teratogenicity, or carcinogenicity. The report must be clearly labeled as a "Safety Report" and must be submitted to the NIH Office of Biotechnology Activities (NIH OBA) and to the local Institutional Biosafety Committee within the timeframes set forth in [Appendix M-I-C-4-b](#).

Principal Investigators should adhere to any other serious adverse event reporting requirements in accordance with federal regulations, state laws, and local institutional policies and procedures, as applicable.

Principal Investigators may delegate to another party, such as a corporate sponsor, the reporting functions set forth in Appendix M, with written notification to the NIH OBA of the delegation and of the name(s), address, telephone and fax numbers of the contact(s). The Principal Investigator is responsible for ensuring that the reporting requirements are fulfilled and will be held accountable for any reporting lapses.

The three alternative mechanisms for reporting serious adverse events to the NIH OBA are: by e-mail to oba@od.nih.gov; by fax to 301-496-9839; or by mail to the Office of Biotechnology Activities, National Institutes of Health, MSC 7985, 6705 Rockledge Drive, Suite 750, Bethesda, Maryland 20892-7985.

Appendix M-I-C-4-a. Safety Reporting: Content and Format

The serious adverse event report must include, but need not be limited to: (1) the date of the event; (2) designation of the report as an initial report or a follow-up report, identification of all safety reports previously filed for the clinical protocol concerning a similar adverse event, and an analysis of the significance of the adverse event in light of previous similar reports; (3) clinical site; (4) the Principal Investigator; (5) NIH Protocol number; (6) FDA's Investigational New Drug (IND) Application number; (7) vector type, e.g., adenovirus; (8) vector subtype, e.g., type 5, relevant deletions; (9) gene delivery method, e.g., *in vivo*, *ex vivo* transduction; (10) route of administration, e.g., intratumoral, intravenous; (11) dosing schedule; (12) a complete description of the event; (13) relevant clinical observations; (14) relevant clinical history; (15) relevant tests that were or are planned to be conducted; (16) date of any treatment of the event; and (17) the suspected cause of the event. These items may be reported by using the recommended Adverse Event Reporting Template available on NIH OBA's web site at: http://oba.od.nih.gov/rdna/adverse_event_oba.html, the FDA MedWatch forms, or other means provided that all of the above elements are specifically included.

Reports from laboratory animal studies as delineated in [Appendix M-I-C-4](#) must be submitted in a narrative format.

Appendix M-I-C-4-b. Safety Reporting: Time frames for Expedited Reports

Any serious adverse event that is fatal or life-threatening, that is unexpected, and associated with the use of the gene transfer product must be reported to the NIH OBA as soon as possible, but not later than 7 calendar days after the sponsor's initial receipt of the information (i.e., at the same time the event must be reported to the FDA).

Serious adverse events that are unexpected and associated with the use of the gene transfer product, but are not fatal or life-threatening, must be reported to the NIH OBA as soon as possible, but not later than 15 calendar days after the sponsor's initial receipt of the information (i.e., at the same time the event must be reported to the FDA).

Changes in this schedule are permitted only where, under the FDA IND regulations [21 CFR 312(c)(3)], changes in this reporting schedule have been approved by the FDA and are reflected in the protocol.

If, after further evaluation, an adverse event initially considered not to be associated with the use of the gene transfer product is subsequently determined to be associated, then the event must be reported to the NIH OBA within 15 days of the determination.

Relevant additional clinical and laboratory data may become available following the initial serious adverse event report. Any follow-up information relevant to a serious adverse event must be reported within 15 calendar days of the sponsor's receipt of the information. If a serious adverse event occurs after the end of a clinical trial and is determined to be associated with the use of the gene transfer product, that event shall be reported to the NIH OBA within 15 calendar days of the determination.

Any finding from tests in laboratory animals that suggests a significant risk for human research participants including reports of mutagenicity, teratogenicity, or carcinogenicity must be reported as soon as possible, but not later than 15 calendar days after the sponsor's initial receipt of the information (i.e., at the same time the event must be reported to the FDA).

Appendix M-I-C-5. Confidentiality

Data submitted in accordance with [Appendix M-I-C](#) that are claimed to be confidential commercial or trade secret information must be clearly labeled as such. Prior to making its determination about the confidentiality of data labeled confidential commercial or trade secret, the NIH will contact the Principal Investigator or delegate to ascertain the basis for the claim and subsequently will notify the Principal Investigator or delegate of its final determination regarding the claim.

If NIH determines that the data so labeled are confidential commercial or trade secret and that their public disclosure would promote an understanding of key scientific or safety issues, the NIH will seek agreement from the appropriate party to release such data. Public discussion of scientific and safety issues raised by data submitted in accordance with [Appendix M-I-C](#) is vital to informing both investigators and human subjects about the safety of gene transfer research.

To protect the privacy of participants in gene transfer research, any serious adverse event or annual reports submitted to NIH OBA must not contain any information that would identify the human research participants.

Appendix M-I-D. Safety Assessment in Human Gene Transfer Research

A working group of the RAC, the NIH Gene Transfer Safety Assessment Board, with staff support from the NIH OBA, will: 1) review in closed session as appropriate safety information from gene transfer trials for the purpose of assessing toxicity and safety data across gene transfer trials; 2) identify significant trends or significant single events; and 3) report significant findings and aggregated trend data to the RAC. It is expected that this process will enhance review of new protocols, improve the development, design, and conduct of human gene transfer trials, promote public understanding and awareness of the safety of human gene transfer research studies, and inform the decision-making of potential trial participants.

Appendix M-II. Description of the Proposal

Responses to this appendix should be provided in the form of either written answers or references to specific sections of the protocol or its appendices. Investigators should indicate the points that are not applicable with a brief explanation. Investigators submitting proposals that employ the same vector systems may refer to preceding documents relating to the vector sequence without having to rewrite such material.

Appendix M-II-A. Objectives and Rationale of the Proposed Research

State concisely the overall objectives and rationale of the proposed study. Provide information on the specific points that relate to whichever type of research is being proposed.

Appendix M-II-A-1. Use of Recombinant DNA for Therapeutic Purposes

For research in which recombinant DNA is transferred in order to treat a disease or disorder (e.g., genetic diseases, cancer, and metabolic diseases), the following questions should be addressed:

Appendix M-II-A-1-a. Why is the disease selected for experimental treatment by means of gene transfer a good candidate for such treatment?

Appendix M-II-A-1-b. Describe the natural history and range of expression of the disease selected for experimental treatment. What objective and/or quantitative measures of disease activity are available? In your view, are the usual effects of the disease predictable enough to allow for meaningful assessment of the results of gene transfer?

Appendix M-II-A-1-c. Is the protocol designed to prevent all manifestations of the disease, to halt the progression of the disease after symptoms have begun to appear, or to reverse manifestations of the disease in seriously ill victims?

Appendix M-II-A-1-d. What alternative therapies exist? In what groups of subjects are these therapies effective? What are their relative advantages and disadvantages as compared with the proposed gene transfer?

Appendix M-II-A-2. Transfer of DNA for Other Purposes

Appendix M-II-A-2-a. Into what cells will the recombinant DNA be transferred? Why is the transfer of recombinant DNA necessary for the proposed research? What questions can be answered by using recombinant DNA?

Appendix M-II-A-2-b. What alternative methodologies exist? What are their relative advantages and disadvantages as compared to the use of recombinant DNA?

Appendix M-II-B. Research Design, Anticipated Risks and Benefits

Appendix M-II-B-1. Structure and Characteristics of the Biological System

Provide a full description of the methods and reagents to be employed for gene delivery and the rationale for their use. The following are specific points to be addressed:

Appendix M-II-B-1-a. What is the structure of the cloned DNA that will be used?

Appendix M-II-B-1-a-(1). Describe the gene (genomic or cDNA), the bacterial plasmid or phage vector, and the delivery vector (if any). Provide complete nucleotide sequence analysis or a detailed restriction enzyme map of the total construct.

Appendix M-II-B-1-a-(2). What regulatory elements does the construct contain (e.g., promoters, enhancers, polyadenylation sites, replication origins, etc.)? From what source are these elements derived? Summarize what is currently known about the regulatory character of each element.

Appendix M-II-B-1-a-(3). Describe the steps used to derive the DNA construct.

Appendix M-II-B-1-b. What is the structure of the material that will be administered to the research participant?

Appendix M-II-B-1-b-(1). Describe the preparation, structure, and composition of the materials that will be given to the human research subject or used to treat the subject's cells: (i) If DNA, what is the purity (both in terms of being a single DNA species and in terms of other contaminants)? What tests have been used and what is the sensitivity of the tests? (ii) If a virus, how is it prepared from the DNA construct? In what cell is the virus grown (any special features)? What medium and serum are used? How is the virus purified? What is its structure and purity? What steps are being taken (and assays used with their sensitivity) to detect and eliminate any contaminating materials (for example, VL30 RNA, other nucleic acids, or proteins) or contaminating viruses (both replication-competent or replication-defective) or other organisms in the cells or serum used for preparation of the virus stock including any contaminants that may have biological effects? (iii) If co-cultivation is employed, what kinds of cells are being used for co-cultivation? What steps are being taken (and assays used with their sensitivity) to detect and eliminate any contaminating materials? Specifically, what tests are being conducted to assess the material to be returned to the subject for the presence of live or killed donor cells or other non-vector materials (for example, VL30 sequences) originating from those cells? (iv) If methods other than those covered by Appendices M-II-B-1 through M-II-B-3, *Research Design, Anticipated Risks and Benefits*, are used to introduce new genetic information into target cells, what steps are being taken to detect and eliminate any contaminating materials? What are possible sources of contamination? What is the sensitivity of tests used to monitor contamination?

Appendix M-II-B-1-b-(2). Describe any other material to be used in preparation of the material to be administered to the human research subject. For example, if a viral vector is proposed, what is the nature of the helper virus or cell line? If carrier particles are to be used, what is the nature of these?

Appendix M-II-B-2. Preclinical Studies, Including Risk-Assessment Studies

Provide results that demonstrate the safety, efficacy, and feasibility of the proposed procedures using animal and/or cell culture model systems, and explain why the model(s) chosen is/are most appropriate.

Appendix M-II-B-2-a. Delivery System

Appendix M-II-B-2-a-(1). What cells are the intended target cells of recombinant DNA? What target cells are to be treated *ex vivo* and returned to the human subject, how will the cells be characterized before and after treatment? What is the theoretical and practical basis for assuming that only the target cells will incorporate the DNA?

Appendix M-II-B-2-a-(2). Is the delivery system efficient? What percentage of the target cells contain the added DNA?

Appendix M-II-B-2-a-(3). How is the structure of the added DNA sequences monitored and what is the sensitivity of the analysis? Is the added DNA extrachromosomal or integrated? Is the added DNA unrearranged?

Appendix M-II-B-2-a-(4). How many copies are present per cell? How stable is the added DNA both in terms of its continued presence and its structural stability?

Appendix M-II-B-2-b. Gene Transfer and Expression

Appendix M-II-B-2-b-(1). What animal and cultured cell models were used in laboratory studies to assess the *in vivo* and *in vitro* efficacy of the gene transfer system? In what ways are these models similar to and different from the proposed human treatment?

Appendix M-II-B-2-b-(2). What is the minimal level of gene transfer and/or expression that is estimated to be necessary for the gene transfer protocol to be successful in humans? How was this level determined?

Appendix M-II-B-2-b-(3). Explain in detail all results from animal and cultured cell model experiments which assess the effectiveness of the delivery system in achieving the minimally required level of gene transfer and expression.

Appendix M-II-B-2-b-(4). To what extent is expression only from the desired gene (and not from the surrounding DNA)? To what extent does the insertion modify the expression of other genes?

Appendix M-II-B-2-b-(5). In what percentage of cells does expression from the added DNA occur? Is the product biologically active? What percentage of normal activity results from the inserted gene?

Appendix M-II-B-2-b-(6). Is the gene expressed in cells other than the target cells? If so, to what extent?

Appendix M-II-B-2-c. Retrovirus Delivery Systems

Appendix M-II-B-2-c-(1). What cell types have been infected with the retroviral vector preparation? Which cells, if any, produce infectious particles?

Appendix M-II-B-2-c-(2). How stable are the retroviral vector and the resulting provirus against loss, rearrangement, recombination, or mutation? What information is available on how much rearrangement or recombination with endogenous or other viral sequences is likely to occur in the human subject's cells? What steps have been taken in designing the vector to minimize instability or variation? What laboratory studies have been performed to check for stability, and what is the sensitivity of the analyses?

Appendix M-II-B-2-c-(3). What laboratory evidence is available concerning potential harmful effects of the transfer (e.g., development of neoplasia, harmful mutations, regeneration of infectious particles, or immune responses)? What steps will be taken in designing the vector to minimize pathogenicity? What laboratory studies have been performed to check for pathogenicity, and what is the sensitivity of the analyses?

Appendix M-II-B-2-c-(4). Is there evidence from animal studies that vector DNA has entered untreated cells, particularly germ-line cells? What is the sensitivity of these analyses?

Appendix M-II-B-2-c-(5). Has a protocol similar to the one proposed for a clinical trial been conducted in non-human primates and/or other animals? What were the results? Specifically, is there any evidence that the retroviral vector has recombined with any endogenous or other viral sequences in the animals?

Appendix M-II-B-2-d. Non-Retrovirus Delivery/Expression Systems

If a non-retroviral delivery system is used, what animal studies have been conducted to determine if there are pathological or other undesirable consequences of the protocol (including insertion of DNA into cells other than those treated, particularly germ-line cells)? How long have the animals been studied after treatment? What safety studies have been conducted? (Include data about the level of sensitivity of such assays.)

Appendix M-II-B-3. Clinical Procedures, Including Research Participant Monitoring

Describe the experimental treatment that will be administered to the human subjects and the diagnostic methods that will be used to monitor the success or failure of the experimental treatment. If previous clinical studies using similar methods have been performed by yourself or others, indicate their relevance to the proposed study. Specifically:

Appendix M-II-B-3-a. Will cells (e.g., bone marrow cells) be removed from human subjects and treated *ex vivo*? If so, describe the type, number, and intervals at which these cells will be removed.

Appendix M-II-B-3-b. Will human subjects be treated to eliminate or reduce the number of cells containing malfunctioning genes (e.g., through radiation or chemotherapy)?

Appendix M-II-B-3-c. What treated cells (or vector/DNA combination) will be given to human subjects? How will the treated cells be administered? What volume of cells will be used? Will there be single or multiple experimental treatments? If so, over what period of time?

Appendix M-II-B-3-d. How will it be determined that new gene sequences have been inserted into the subject's cells and if these sequences are being expressed? Are these cells limited to the intended target cell populations? How sensitive are these analyses?

Appendix M-II-B-3-e. What studies will be conducted to assess the presence and effects of the contaminants?

Appendix M-II-B-3-f. What are the clinical endpoints of the study? Are there objectives and quantitative measurements to assess the natural history of the disease? Will such measurements be used in human subject follow-up? How will subjects be monitored to assess specific effects of the treatment on the disease? What is the sensitivity of the analyses? How frequently will follow-up studies be conducted? How long will follow-up continue?

Appendix M-II-B-3-g. What are the major beneficial and adverse effects of the experimental treatment that you anticipate? What measures will be taken in an attempt to control or reverse these adverse effects if they occur? Compare the probability and magnitude of deleterious consequences from the disease if recombinant DNA transfer is not used.

Appendix M-II-B-3-h. If a treated human subject dies, what special post-mortem studies will be performed?

Appendix M-II-B-4. Public Health Considerations

Describe any potential benefits and hazards of the proposed gene transfer to persons other than the human subjects receiving the experimental treatment. Specifically:

Appendix M-II-B-4-a. On what basis are potential public health benefits or hazards postulated?

Appendix M-II-B-4-b. Is there a significant possibility that the added DNA will spread from the human subject to other persons or to the environment?

Appendix M-II-B-4-c. What precautions will be taken against such spread (e.g., subjects sharing a room, health-care workers, or family members)?

Appendix M-II-B-4-d. What measures will be undertaken to mitigate the risks, if any, to public health?

Appendix M-II-B-4-e. In light of possible risks to offspring, including vertical transmission, will birth control measures be recommended to subjects? Are such concerns applicable to health care personnel?

Appendix M-II-B-5. Qualifications of Investigators and Adequacy of Laboratory and Clinical Facilities

Indicate the relevant training and experience of the personnel who will be involved in the preclinical studies and clinical administration of recombinant DNA. Describe the laboratory and clinical facilities where the proposed study will be performed. Specifically:

Appendix M-II-B-5-a. What professional personnel (medical and nonmedical) will be involved in the proposed study and what is their relevant expertise? Provide a two-page curriculum vitae for each key professional person in biographical sketch format (see [Appendix M-I-A, Requirements for Protocol Submission](#)).

Appendix M-II-B-5-b. At what hospital or clinic will the experimental treatment be given? Which facilities of the hospital or clinic will be especially important for the proposed study? Will subjects occupy regular hospital beds or clinical research center beds? Where will subjects reside during the follow-up period? What special arrangements will be made for the comfort and consideration of the research participants. Will the research institution designate an ombudsman, patient care representative, or other individual to help protect the rights and welfare of the research participant?

Appendix M-II-C. Selection of the Human Subjects

Estimate the number of human subjects to be involved in the proposed study. Describe recruitment procedures and eligibility requirements, paying particular attention to whether these procedures and requirements are fair and equitable. Specifically:

Appendix M-II-C-1. How many subjects do you plan to involve in the proposed study?

Appendix M-II-C-2. How many eligible subjects do you anticipate being able to identify each year?

Appendix M-II-C-3. What recruitment procedures do you plan to use?

Appendix M-II-C-4. What selection criteria do you plan to employ? What are the exclusion and inclusion criteria for the study?

Appendix M-II-C-5. How will subjects be selected if it is not possible to include all who desire to participate?

Appendix M-III. Informed Consent

In accordance with the Protection of Human Subjects (45 CFR Part 46), investigators should indicate how subjects will be informed about the proposed study and the manner in which their consent will be solicited. They should indicate how the Informed Consent document makes clear the special requirements of gene transfer research. If a proposal involves children, special attention should be paid to the Protection of Human Subjects (45 CFR Part 46), Subpart D, Additional Protections for Children Involved as Subjects in Research.

Appendix M-III-A. Communication About the Study to Potential Participants

Appendix M-III-A-1. Which members of the research group and/or institution will be responsible for contacting potential participants and for describing the study to them? What procedures will be used to avoid possible conflicts of interest if the investigator is also providing medical care to potential subjects?

Appendix M-III-A-2. How will the major points covered in [Appendix M-II, Description of Proposal](#), be disclosed to potential participants and/or their parents or guardians in language that is understandable to them?

Appendix M-III-A-3. What is the length of time that potential participants will have to make a decision about their participation in the study?

Appendix M-III-A-4. If the study involves pediatric or mentally handicapped subjects, how will the assent of each person be obtained?

Appendix M-III-B. Informed Consent Document

Submission of a human gene transfer experiment to NIH OBA must include a copy of the proposed informed consent document. A separate Informed Consent document should be used for the gene transfer portion of a research project when gene transfer is used as an adjunct in the study of another technique, e.g., when a gene is used as a "marker" or to enhance the power of immunotherapy for cancer.

Because of the relative novelty of the procedures that are used, the potentially irreversible consequences of the procedures performed, and the fact that many of the potential risks remain undefined, the Informed Consent document should include the following specific information in addition to any requirements of the DHHS regulations for the Protection of Human Subjects (45 CFR 46). Indicate if each of the specified items appears in the Informed Consent document or, if not included in the Informed Consent document, how those items will be presented to potential subjects. Include an explanation if any of the following items are omitted from the consent process or the Informed Consent document.

Appendix M-III-B-1. General Requirements of Human Subjects Research

Appendix M-III-B-1-a. Description/Purpose of the Study

The subjects should be provided with a detailed explanation in non-technical language of the purpose of the study and the procedures associated with the conduct of the proposed study, including a description of the gene transfer component.

Appendix M-III-B-1-b. Alternatives

The Informed Consent document should indicate the availability of therapies and the possibility of other investigational interventions and approaches.

Appendix M-III-B-1-c. Voluntary Participation

The subjects should be informed that participation in the study is voluntary and that failure to participate in the study or withdrawal of consent will not result in any penalty or loss of benefits to which the subjects are otherwise entitled.

Appendix M-III-B-1-d. Benefits

The subjects should be provided with an accurate description of the possible benefits, if any, of participating in the proposed study. For studies that are not reasonably expected to provide a therapeutic benefit to subjects, the Informed Consent document should clearly state that no direct clinical benefit to subjects is expected to occur as a result of participation in the study, although knowledge may be gained that may benefit others.

Appendix M-III-B-1-e. Possible Risks, Discomforts, and Side Effects

There should be clear itemization in the Informed Consent document of types of adverse experiences, their relative severity, and their expected frequencies. For consistency, the following definitions are suggested: side effects that are listed as mild should be ones which do not require a therapeutic intervention; moderate side effects require an intervention; and severe side effects are potentially fatal or life-threatening, disabling, or require prolonged hospitalization.

If verbal descriptors (e.g., "rare," "uncommon," or "frequent") are used to express quantitative information regarding risk, these terms should be explained.

The Informed Consent document should provide information regarding the approximate number of people who have previously received the genetic material under study. It is necessary to warn potential subjects that, for genetic materials previously used in relatively few or no humans, unforeseen risks are possible, including ones that could be severe.

The Informed Consent document should indicate any possible adverse medical consequences that may occur if the subjects withdraw from the study once the study has started.

Appendix M-III-B-1-f. Costs

The subjects should be provided with specific information about any financial costs associated with their participation in the protocol and in the long-term follow-up to the protocol that are not covered by the investigators or the institution involved.

Subjects should be provided an explanation about the extent to which they will be responsible for any costs for medical treatment required as a result of research-related injury.

Appendix M-III-B-2. Specific Requirements of Gene Transfer Research**Appendix M-III-B-2-a. Reproductive Considerations**

To avoid the possibility that any of the reagents employed in the gene transfer research could cause harm to a fetus/child, subjects should be given information concerning possible risks and the need for contraception by males and females during the active phase of the study. The period of time for the use of contraception should be specified.

The inclusion of pregnant or lactating women should be addressed.

Appendix M-III-B-2-b. Long-Term Follow-Up

To permit evaluation of long-term safety and efficacy of gene transfer, the prospective subjects should be informed that they are expected to cooperate in long-term follow-up that extends beyond the active phase of the study. The Informed Consent document should include a list of persons who can be contacted in the event that questions arise during the follow-up period. The investigator should request that subjects continue to provide a current address and telephone number.

The subjects should be informed that any significant findings resulting from the study will be made known in a timely manner to them and/or their parent or guardian including new information about the experimental procedure, the harms and benefits experienced by other individuals involved in the study, and any long-term effects that have been observed.

Appendix M-III-B-2-c. Request for Autopsy

To obtain vital information about the safety and efficacy of gene transfer, subjects should be informed that at the time of death, no matter what the cause, permission for an autopsy will be requested of their families. Subjects should be asked to advise their families of the request and of its scientific and medical importance.

Appendix M-III-B-2-d. Interest of the Media and Others in the Research

To alert subjects that others may have an interest in the innovative character of the protocol and in the status of the treated subjects, the subjects should be informed of the following: (i) that the institution and investigators will make efforts to provide protection from the media in an effort to protect the participants' privacy, and (ii) that representatives of applicable Federal agencies (e.g., the [National Institutes of Health](#) and the [Food and Drug Administration](#)), representatives of collaborating institutions, vector suppliers, etc., will have access to the subjects' medical records.

Appendix M-IV. Privacy

Indicate what measures will be taken to protect the privacy of subjects and their families as well as maintain the confidentiality of research data. These measures should help protect the confidentiality of information that could directly or indirectly identify study participants.

Appendix M-IV-A. What provisions will be made to honor the wishes of individual human subjects (and the parents or guardians of pediatric or mentally handicapped subjects) as to whether, when, or how the identity of a subject is publicly disclosed.

Appendix M-IV-B. What provisions will be made to maintain the confidentiality of research data, at least in cases where data could be linked to individual subjects?

Appendix M-V. Special Issues

Although the following issues are beyond the normal purview of local Institutional Review Boards, investigators should respond to the following questions:

Appendix M-V-A. What steps will be taken, consistent with Appendix M-IV, *Privacy*, to ensure that accurate and appropriate information is made available to the public with respect to such public concerns as may arise from the proposed study?

Appendix M-V-B. Do you or your funding sources intend to protect under patent or trade secret laws either the products or the procedures developed in the proposed study? If so, what steps will be taken to permit as full communication as possible among investigators and clinicians concerning research methods and results?

Appendix M-VI. Footnotes of Appendix M

Appendix M-VI-A. Human studies in which induction or enhancement of an immune response to a vector-encoded microbial immunogen is the major goal, such an immune response has been demonstrated in model systems, and the persistence of the vector-encoded immunogen is not expected, are exempt from [Appendix M-I, Requirements for Protocol Submission, Review and Reporting – Human Gene Transfer Experiments](#).

APPENDIX P. PHYSICAL AND BIOLOGICAL CONTAINMENT FOR RECOMBINANT DNA RESEARCH INVOLVING PLANTS

Appendix P specifies physical and biological containment conditions and practices suitable to the greenhouse conduct of experiments involving recombinant DNA-containing plants, plant-associated microorganisms, and small animals. All provisions of the *NIH Guidelines* apply to plant research activities with the following modifications:

Appendix P shall supersede [Appendix G \(Physical Containment\)](#) when the research plants are of a size, number, or have growth requirements that preclude the use of containment conditions described in [Appendix G](#). The plants covered in Appendix P include but are not limited to mosses, liverworts, macroscopic algae, and vascular plants including terrestrial crops, forest, and ornamental species.

Plant-associated microorganisms include viroids, virusoids, viruses, bacteria, fungi, protozoans, certain small algae, and microorganisms that have a benign or beneficial association with plants, such as certain *Rhizobium* species and microorganisms known to cause plant diseases. The appendix applies to microorganisms which are being modified with the objective of fostering an association with plants.

Plant-associated small animals include those arthropods that: (i) are in obligate association with plants, (ii) are plant pests, (iii) are plant pollinators, or (iv) transmit plant disease agents, as well as other small animals such as nematodes for which tests of biological properties necessitate the use of plants. Microorganisms associated with such small animals (e.g., pathogens or symbionts) are included.

The Institutional Biosafety Committee shall include at least one individual with expertise in plant, plant pathogen, or plant pest containment principles when experiments utilizing Appendix P require prior approval by the Institutional Biosafety Committee.

Appendix P-I. General Plant Biosafety Levels

Appendix P-I-A. The principal purpose of plant containment is to avoid the unintentional transmission of a recombinant DNA-containing plant genome, including nuclear or organelle hereditary material or release of recombinant DNA-derived organisms associated with plants.

Appendix P-I-B. The containment principles are based on the recognition that the organisms that are used pose no health threat to humans or higher animals (unless deliberately modified for that purpose), and that the containment conditions minimize the possibility of an unanticipated deleterious effect on organisms and ecosystems outside of the experimental facility, e.g., the inadvertent spread of a serious pathogen from a greenhouse to a local agricultural crop or the unintentional introduction and establishment of an organism in a new ecosystem.

Appendix P-I-C. Four biosafety levels, referred to as Biosafety Level (BL) 1 - Plants (P), BL2-P, BL3-P, and BL4-P, are established in [Appendix P-II, Physical Containment Levels](#). The selection of containment levels required for research involving recombinant DNA molecules in plants or associated with plants is specified in [Appendix P-III, Biological Containment Practices](#). These biosafety levels are described in Appendix P-II, *Physical Containment Levels*. This appendix describes greenhouse practices and special greenhouse facilities for physical containment.

Appendix P-I-D. BL1-P through BL4-P are designed to provide differential levels of biosafety for plants in the absence or presence of other experimental organisms that contain recombinant DNA. These biosafety levels, in conjunction with biological containment conditions described in [Appendix P-III, *Biological Containment Practices*](#), provide flexible approaches to ensure the safe conduct of research.

Appendix P-I-E. For experiments in which plants are grown at the BL1 through BL4 laboratory settings, containment practices shall be followed as described in [Appendix G, *Physical Containment*](#). These containment practices include the use of plant tissue culture rooms, growth chambers within laboratory facilities, or experiments performed on open benches. Additional biological containment practices should be added by the Greenhouse Director or Institutional Biosafety Committee as necessary (see [Appendix P-III, *Biological Containment Practices*](#)), if botanical reproductive structures are produced that have the potential of being released.

Appendix P-II. Physical Containment Levels

Appendix P-II-A. Biosafety Level 1 - Plants (BL1-P)

Appendix P-II-A-1. Standard Practices (BL1-P)

Appendix P-II-A-1-a. Greenhouse Access (BL1-P)

Appendix P-II-A-1-a-(1). Access to the greenhouse shall be limited or restricted, at the discretion of the Greenhouse Director, when experiments are in progress.

Appendix P-II-A-1-a-(2). Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL1-P greenhouse practices and procedures. All procedures shall be performed in accordance with accepted greenhouse practices that are appropriate to the experimental organism.

Appendix P-II-A-1-b. Records (BL1-P)

Appendix P-II-A-1-b-(1). A record shall be kept of experiments currently in progress in the greenhouse facility.

Appendix P-II-A-1-c. Decontamination and Inactivation (BL1-P)

Appendix P-II-A-1-c-(1). Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.

Appendix P-II-A-1-d. Control of Undesired Species and Motile Macroorganisms (BL1-P)

Appendix P-II-A-1-d-(1). A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens), by methods appropriate to the organisms and in accordance with applicable state and Federal laws.

Appendix P-II-A-1-d-(2). Arthropods and other motile macroorganisms shall be housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility.

Appendix P-II-A-1-e. Concurrent Experiments Conducted in the Greenhouse (BL1-P)

Appendix P-II-A-1-e-(1). Experiments involving other organisms that require a containment level lower than BL1-P may be conducted in the greenhouse concurrently with experiments that require BL1-P containment, provided that all work is conducted in accordance with BL1-P greenhouse practices.

Appendix P-II-A-2. Facilities (BL1-P)

Appendix P-II-A-2-a. Definitions (BL1-P)

Appendix P-II-A-2-a-(1). The term "greenhouse" refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.

Appendix P-II-A-2-a-(2). The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas, and is considered part of the confinement area.

Appendix P-II-A-2-b. Greenhouse Design (BL1-P)

Appendix P-II-A-2-b-(1). The greenhouse floor may be composed of gravel or other porous material. At a minimum, impervious (e.g., concrete) walkways are recommended.

Appendix P-II-A-2-b-(2). Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to contain or exclude pollen, microorganisms, or small flying animals (e.g., arthropods and birds); however, screens are recommended.

Appendix P-II-B. Biosafety Level 2 - Plants (BL2-P)

Appendix P-II-B-1. Standard Practices (BL2-P)

Appendix P-II-B-1-a. Greenhouse Access (BL2-P)

Appendix P-II-B-1-a-(1). Access to the greenhouse shall be limited or restricted, at the discretion of the Greenhouse Director, to individuals directly involved with the experiments when they are in progress.

Appendix P-II-B-1-a-(2). Personnel shall be required to read and follow instructions on BL2-P practices and procedures. All procedures shall be conducted in accordance with accepted greenhouse practices that are appropriate to the experimental organisms.

Appendix P-II-B-1-b. Records (BL2-P)

Appendix P-II-B-1-b-(1). A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility.

Appendix P-II-B-1-b-(2). A record shall be kept of experiments currently in progress in the greenhouse facility.

Appendix P-II-B-1-b-(3). The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Greenhouse Director, Institutional Biosafety Committee, NIH/OBA and other appropriate authorities immediately (if applicable). Reports to the NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Documentation of any such accident shall be prepared and maintained.

Appendix P-II-B-1-c. Decontamination and Inactivation (BL2-P)

Appendix P-II-B-1-c-(1). Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.

Appendix P-II-B-1-c-(2). Decontamination of run-off water is not necessarily required. If part of the greenhouse is composed of gravel or similar material, appropriate treatments should be made periodically to eliminate, or render inactive, any organisms potentially entrapped by the gravel.

Appendix P-II-B-1-d. Control of Undesired Species and Motile Macroorganisms (BL2-P)

Appendix P-II-B-1-d-(1). A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens) by methods appropriate to the organisms and in accordance with applicable state and Federal laws.

Appendix P-II-B-1-d-(2). Arthropods and other motile macroorganisms shall be housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility.

Appendix P-II-B-1-e. Concurrent Experiments Conducted in the Greenhouse (BL2-P)

Appendix P-II-B-1-e-(1). Experiments involving other organisms that require a containment level lower than BL2-P may be conducted in the greenhouse concurrently with experiments that require BL2-P containment provided that all work is conducted in accordance with BL2-P greenhouse practices.

Appendix P-II-B-1-f. Signs (BL2-P)

Appendix P-II-B-1-f-(1). A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) the name of the responsible individual, (ii) the plants in use, and (iii) any special requirements for using the area.

Appendix P-II-B-1-f-(2). If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence shall be indicated on a sign posted on the greenhouse access doors.

Appendix P-II-B-1-f-(3). If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.

Appendix P-II-B-1-g. Transfer of Materials (BL2-P)

Appendix P-II-B-1-g-(1). Materials containing experimental microorganisms, which are brought into or removed from the greenhouse facility in a viable or intact state, shall be transferred in a closed non-breakable container.

Appendix P-II-B-1-h. Greenhouse Practices Manual (BL2-P)

Appendix P-II-B-1-h-(1). A greenhouse practices manual shall be prepared or adopted. This manual shall: (i) advise personnel of the potential consequences if such practices are not followed, and (ii) outline contingency plans to be implemented in the event of the unintentional release of organisms.

Appendix P-II-B-2. Facilities (BL2-P)

Appendix P-II-B-2-a. Definitions (BL2-P)

Appendix P-II-B-2-a-(1). The term "greenhouse" refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.

Appendix P-II-B-2-a-(2). The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas and is considered part of the confinement area.

Appendix P-II-B-2-b. Greenhouse Design (BL2-P)

Appendix P-II-B-2-b-(1). A greenhouse floor composed of an impervious material. Concrete is recommended, but gravel or other porous material under benches is acceptable unless propagules of experimental organisms are readily disseminated through soil. Soil beds are acceptable unless propagules of experimental organisms are readily disseminated through soil.

Appendix P-II-B-2-b-(2). Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to exclude pollen or microorganisms; however, screens are required to exclude small flying animals (e.g., arthropods and birds).

Appendix P-II-B-2-c. Autoclaves (BL2-P)

Appendix P-II-B-2-c-(1). An autoclave shall be available for the treatment of contaminated greenhouse materials.

Appendix P-II-B-2-d. Supply and Exhaust Air Ventilation Systems (BL2-P)

Appendix P-II-B-2-d-(1). If intake fans are used, measures shall be taken to minimize the ingress of arthropods. Louvers or fans shall be constructed such that they can only be opened when the fan is in operation.

Appendix P-II-B-2-e. Other (BL2-P)

Appendix P-II-B-2-e-(1). BL2-P greenhouse containment requirements may be satisfied by using a growth chamber or growth room within a building provided that the external physical structure limits access and escape of microorganisms and macroorganisms in a manner that satisfies the intent of the foregoing clauses.

Appendix P-II-C. Biosafety Level 3 - Plants (BL3-P)**Appendix P-II-C-1. Standard Practices (BL3-P)****Appendix P-II-C-1-a. Greenhouse Access (BL3-P)**

Appendix P-II-C-1-a-(1). Authorized entry into the greenhouse shall be restricted to individuals who are required for program or support purposes. The Greenhouse Director shall be responsible for assessing each circumstance and determining those individuals who are authorized to enter the greenhouse facility.

Appendix P-II-C-1-a-(2). Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL3-P practices and procedures. All procedures shall be conducted in accordance with accepted greenhouse practices that are appropriate to the experimental organisms.

Appendix P-II-C-1-b. Records (BL3-P)

Appendix P-II-C-1-b-(1). A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility.

Appendix P-II-C-1-b-(2). A record shall be kept of experiments currently in progress in the greenhouse facility.

Appendix P-II-C-1-b-(3). The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Biological Safety Officer, Greenhouse Director, Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities immediately (if applicable). Reports to the NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Documentation of any such accident shall be prepared and maintained.

Appendix P-II-C-1-c. Decontamination and Inactivation (BL3-P)

Appendix P-II-C-1-c-(1). All experimental materials shall be sterilized in an autoclave or rendered biologically inactive by appropriate methods before disposal, except those that are to remain in a viable or intact state for experimental purposes; including water that comes in contact with experimental microorganisms or with material exposed to such microorganisms, and contaminated equipment and supplies.

Appendix P-II-C-1-d. Control of Undesired Species and Motile Macroorganisms (BL3-P)

Appendix P-II-C-1-d-(1). A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens) by methods appropriate to the organisms and in accordance with applicable state and Federal laws.

Appendix P-II-C-1-d-(2). Arthropods and other motile macroorganisms shall be housed in appropriate cages. When appropriate to the organism, experiments shall be conducted within cages designed to contain the motile organisms.

Appendix P-II-C-1-e. Concurrent Experiments Conducted in the Greenhouse (BL3-P)

Appendix P-II-C-1-e-(1). Experiments involving organisms that require a containment level lower than BL3-P may be conducted in the greenhouse concurrently with experiments that require BL3-P containment provided that all work is conducted in accordance with BL3-P greenhouse practices.

Appendix P-II-C-1-f. Signs (BL3-P)

Appendix P-II-C-1-f-(1). A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) the name of the responsible individual, (ii) the plants in use, and (iii) any special requirements for using the area.

Appendix P-II-C-1-f-(2). If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence should be indicated on a sign posted on the greenhouse access doors.

Appendix P-II-C-1-f-(3). If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.

Appendix P-II-C-1-g. Transfer of Materials (BL3-P)

Appendix P-II-C-1-g-(1). Experimental materials that are brought into or removed from the greenhouse facility in a viable or intact state shall be transferred to a non-breakable sealed secondary container. At the time of transfer, if the same plant species, host, or vector are present within the effective dissemination distance of propagules of the experimental organism, the surface of the secondary container shall be decontaminated. Decontamination may be accomplished by passage through a chemical disinfectant or fumigation chamber or by an alternative procedure that has demonstrated effective inactivation of the experimental organism.

Appendix P-II-C-1-h. Greenhouse Practices Manual (BL3-P)

Appendix P-II-C-1-h-(1). A greenhouse practices manual shall be prepared or adopted. This manual shall: (i) advise personnel of the potential consequences if such practices are not followed, and (ii) outline contingency plans to be implemented in the event of the unintentional release of organisms with recognized potential for serious detrimental impact.

Appendix P-II-C-1-i. Protective Clothing (BL3-P)

Appendix P-II-C-1-i-(1). Disposable clothing (e.g., solid front or wrap-around gowns, scrub suits, or other appropriate clothing) shall be worn in the greenhouse if deemed necessary by the Greenhouse Director because of potential dissemination of the experimental microorganisms.

Appendix P-II-C-1-i-(2). Protective clothing shall be removed before exiting the greenhouse and decontaminated prior to laundering or disposal.

Appendix P-II-C-1-j. Other (BL3-P)

Appendix P-II-C-1-j-(1). Personnel are required to thoroughly wash their hands upon exiting the greenhouse.

Appendix P-II-C-1-j-(2). All procedures shall be performed carefully to minimize the creation of aerosols and excessive splashing of potting material/soil during watering, transplanting, and all experimental manipulations.

Appendix P-II-C-2. Facilities (BL3-P)**Appendix P-II-C-2-a. Definitions (BL3-P)**

Appendix P-II-C-2-a-(1). The term "greenhouse" refers to a structure with walls, roof, and floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.

Appendix P-II-C-2-a-(2). The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas, and is considered part of the confinement area. The need to maintain negative pressure should be considered when constructing or renovating the greenhouse.

Appendix P-II-C-2-b. Greenhouse Design (BL3-P)

Appendix P-II-C-2-b-(1). The greenhouse floor shall be composed of concrete or other impervious material with provision for collection and decontamination of liquid run-off.

Appendix P-II-C-2-b-(2). Windows shall be closed and sealed. All glazing shall be resistant to breakage (e.g., double-pane tempered glass or equivalent).

Appendix P-II-C-2-b-(3). The greenhouse shall be a closed self-contained structure with a continuous covering that is separated from areas that are open to unrestricted traffic flow. The minimum requirement for greenhouse entry shall be passage through two sets of self-closing locking doors.

Appendix P-II-C-2-b-(4). The greenhouse facility shall be surrounded by a security fence or protected by equivalent security measures.

Appendix P-II-C-2-b-(5). Internal walls, ceilings, and floors shall be resistant to penetration by liquids and chemicals to facilitate cleaning and decontamination of the area. All penetrations into these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

Appendix P-II-C-2-b-(6). Bench tops and other work surfaces should have seamless surfaces that are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix P-II-C-2-b-(7). The greenhouse contains a foot, elbow, or automatically operated sink, which is located near the exit door for hand washing.

Appendix P-II-C-2-c. Autoclaves (BL3-P)

Appendix P-II-C-2-c-(1). An autoclave shall be available for decontaminating materials within the greenhouse facility. A double-door autoclave is recommended (not required) for the decontamination of materials passing out of the greenhouse facility.

Appendix P-II-C-2-d. Supply and Exhaust Air Ventilation Systems (BL3-P)

Appendix P-II-C-2-d-(1). An individual supply and exhaust air ventilation system shall be provided. The system maintains pressure differentials and directional airflow, as required, to assure inward (or zero) airflow from areas outside of the greenhouse.

Appendix P-II-C-2-d-(2). The exhaust air from the greenhouse facility shall be filtered through high efficiency particulate air-HEPA filters and discharged to the outside. The filter chambers shall be designed to allow *in situ* decontamination before filters are removed and to facilitate certification testing after they are replaced. Air filters shall be 80-85% average efficiency by the American Society of Heating, Refrigerating, and Air Conditioning Engineers (ASHRAE) Standard 52-68 test method using atmosphere dust. Air supply fans shall be equipped with a back-flow damper that closes when the air supply fan is off. Alternatively, a HEPA filter may be used on the air supply system instead of the filters and damper. The supply and exhaust airflow shall be interlocked to assure inward (or zero) airflow at all times.

Appendix P-II-C-2-e. Other (BL3-P)

Appendix P-II-C-2-e-(1). BL3-P greenhouse containment requirements may be satisfied using a growth chamber or growth room within a building provided that the location, access, airflow patterns, and provisions for decontamination of experimental materials and supplies meet the intent of the foregoing clauses.

Appendix P-II-C-2-e-(2). Vacuum lines shall be protected with high efficiency particulate air/HEPA or equivalent filters and liquid disinfectant traps.

Appendix P-II-D. Biosafety Level 4 - Plants (BL4-P)**Appendix P-II-D-1. Standard Practices (BL4-P)****Appendix P-II-D-1-a. Greenhouse Access (BL4-P)**

Appendix P-II-D-1-a-(1). Authorized entry into the greenhouse shall be restricted to individuals who are required for program or support purposes. The Greenhouse Director shall be responsible for assessing each circumstance and determining those individuals who are authorized to enter the greenhouse facility or work in the greenhouse during experiments.

Appendix P-II-D-1-a-(2). Access shall be managed by the Greenhouse Director, Biological Safety Officer, or other individual responsible for physical security of the greenhouse facility; and access limited by means of secure, locked doors.

Appendix P-II-D-1-a-(3). Prior to entering, individuals shall be advised of the potential environmental hazards and instructed on appropriate safeguards for ensuring environmental safety. Individuals authorized to enter the greenhouse facility shall comply with the instructions and all other applicable entry/exit procedures.

Appendix P-II-D-1-a-(4). Personnel shall enter and exit the greenhouse facility only through the clothing change and shower rooms and shall shower each time they exit the greenhouse facility. Personnel shall use the airlocks to enter or exit the laboratory only in an emergency. In the event of an emergency, every reasonable effort should be made to prevent the possible transport of viable propagules from containment.

Appendix P-II-D-1-a-(5). Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL4-P practices and procedures.

Appendix P-II-D-1-b. Records (BL4-P)

Appendix P-II-D-1-b-(1). A record shall be kept of all experimental materials brought into or removed from the greenhouse.

Appendix P-II-D-1-b-(2). A record shall be kept of experiments currently in progress in the greenhouse facility.

Appendix P-II-D-1-b-(3). A record shall be kept of all personnel entering and exiting the greenhouse facility, including the date and time of each entry.

Appendix P-II-D-1-b-(4). The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Biological Safety Officer, Greenhouse Director, Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities immediately (if applicable). Reports to the NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Documentation of any such accident shall be prepared and maintained.

Appendix P-II-D-1-c. Decontamination and Inactivation (BL4-P)

Appendix P-II-D-1-c-(1). All materials, except for those that are to remain in a viable or intact state for experimental purposes, shall be autoclaved prior to removal from the maximum containment greenhouse. Equipment or material that could be damaged by high temperatures or steam shall be decontaminated by alternative methods (e.g., gas or vapor sterilization) in an airlock or chamber designed for this purpose.

Appendix P-II-D-1-c-(2). Water that comes in contact with experimental microorganisms or with material exposed to such microorganisms (e.g., run-off from watering plants) shall be collected and decontaminated before disposal.

Appendix P-II-D-1-c-(3). Standard microbiological procedures shall be followed for decontamination of equipment and materials. Spray or liquid waste or rinse water from containers used to apply the experimental microorganisms shall be decontaminated before disposal.

Appendix P-II-D-1-d. Control of Undesired Species and Motile Macroorganisms (BL4-P)

Appendix P-II-D-1-d-(1). A chemical control program shall be implemented to eliminate undesired pests and pathogens in accordance with applicable state and Federal laws.

Appendix P-II-D-1-d-(2). Arthropods and other motile macroorganisms used in conjunction with experiments requiring BL4-P level physical containment shall be housed in appropriate cages. When appropriate to the organism, experiments shall be conducted within cages designed to contain the motile organisms.

Appendix P-II-D-1-e. Concurrent Experiments Conducted in the Greenhouse (BL4-P)

Appendix P-II-D-1-e-(1). Experiments involving organisms that require a containment level lower than BL4-P may be conducted in the greenhouse concurrently with experiments that require BL4-P containment provided that all work is conducted in accordance with BL4-P greenhouse practices. When the experimental microorganisms in use require a containment level lower than BL4-P, greenhouse practices reflect the level of containment required by the highest containment level microorganisms being tested.

Appendix P-II-D-1-f. Signs (BL4-P)

Appendix P-II-D-1-f-(1). A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) the name of the responsible individual, (ii) the plants in use, and (iii) any special requirements for using the area.

Appendix P-II-D-1-f-(2). If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence shall be indicated by a sign posted on the greenhouse access doors.

Appendix P-II-D-1-f-(3). If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.

Appendix P-II-D-1-g. Transfer of Materials (BL4-P)

Appendix P-II-D-1-g-(1). Experimental materials that are brought into or removed from the greenhouse in a viable or intact state shall be transferred to a non-breakable, sealed, primary container then enclosed in a non-breakable, sealed secondary container. These containers shall be removed from the greenhouse facility through a chemical disinfectant, fumigation chamber, or an airlock designed for this purpose.

Appendix P-II-D-1-g-(2). Supplies and materials shall be brought into the greenhouse facility through a double-door autoclave, fumigation chamber, or airlock that is appropriately decontaminated between each use. After securing the outer doors, personnel within the greenhouse facility shall retrieve the materials by opening the interior door of the autoclave, fumigation chamber, or airlock. These doors shall be secured after the materials are brought into the greenhouse facility.

Appendix P-II-D-1-h. Greenhouse Practices Manual (BL4-P)

Appendix P-II-D-1-h-(1). A greenhouse practices manual shall be prepared or adopted. This manual shall include contingency plans to be implemented in the event of the unintentional release of experimental organisms.

Appendix P-II-D-1-i. Protective Clothing (BL4-P)

Appendix P-II-D-1-i-(1). Street clothing shall be removed in the outer clothing change room. Complete laboratory clothing (may be disposable) including undergarments, pants, and shirts, jump suits, shoes, and hats shall be provided and worn by all personnel entering the greenhouse facility.

Appendix P-II-D-1-i-(2). Personnel shall remove laboratory clothing when exiting the greenhouse facility and before entering the shower area. This clothing shall be stored in a locker or hamper in the inner change room.

Appendix P-II-D-1-i-(3). All laboratory clothing shall be autoclaved before laundering.

Appendix P-II-D-2. Facilities (BL4-P)**Appendix P-II-D-2-a. Greenhouse Design (BL4-P)**

Appendix P-II-D-2-a-(1). The maximum containment greenhouse facility shall consist of a separate building or a clearly demarcated and isolated area within a building. The need to maintain negative pressure should be considered when constructing or renovating the greenhouse facility.

Appendix P-II-D-2-a-(2). Outer and inner change rooms, separated by a shower, shall be provided for personnel entering and exiting the greenhouse facility.

Appendix P-II-D-2-a-(3). Windows shall be closed and sealed. All glazing shall be resistant to breakage (e.g., double-pane tempered glass or equivalent).

Appendix P-II-D-2-a-(4). Access doors to the greenhouse shall be self-closing and locking.

Appendix P-II-D-2-a-(5). The greenhouse facility shall be surrounded by a security fence or protected by equivalent security measures.

Appendix P-II-D-2-a-(6). The walls, floors, and ceilings of the greenhouse shall be constructed to form a sealed internal shell that facilitates fumigation and is animal and arthropod-proof. These internal surfaces shall be resistant to penetration and degradation by liquids and chemicals to facilitate cleaning and decontamination of the area. All penetrations into these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

Appendix P-II-D-2-a-(7). Bench tops and other work surfaces shall have seamless surfaces impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix P-II-D-2-a-(8). A double-door autoclave, fumigation chamber, or ventilated airlock shall be provided for passage of all materials, supplies, or equipment that are not brought into the greenhouse facility through the change room.

Appendix P-II-D-2-b. Autoclaves (BL4-P)

Appendix P-II-D-2-b-(1). A double-door autoclave shall be provided for the decontamination of materials removed from the greenhouse facility. The autoclave door, which opens to the area external to the greenhouse facility, shall be sealed to the outer wall and automatically controlled so that it can only be opened upon completion of the sterilization cycle.

Appendix P-II-D-2-c. Supply and Exhaust Air Ventilation Systems (BL4-P)

Appendix P-II-D-2-c-(1). An individual supply and exhaust air ventilation system shall be provided. The system shall maintain pressure differentials and directional airflow as required to assure inward (or zero) airflow from areas outside of the greenhouse. Differential pressure transducers shall be used to sense pressure levels. If a system malfunctions, the transducers shall sound an alarm. A backup source of power should be considered. The supply and exhaust airflow shall be interlocked to assure inward (or zero) airflow at all times. The integrity of the greenhouse shall have an air leak rate (decay rate) not to exceed 7 percent per minute (logarithm of pressure against time) over a 20-minute period at 2 inches of water gauge pressure. Nominally, this is 0.05 inches of water gauge pressure loss in 1 minute at 2 inches water gauge pressure.

Appendix P-II-D-2-c-(2). Exhaust air from the greenhouse facility shall be filtered through high efficiency particulate air/HEPA filters and discharged to the outside and dispersed away from occupied buildings and air intakes. Filter chambers shall be designed to allow *in situ* decontamination before filters are removed and to facilitate certification testing after they are replaced. HEPA filters shall be provided to treat air supplied to the greenhouse facility. HEPA filters shall be certified annually.

Appendix P-II-D-2-d. Other (BL4-P)

Appendix P-II-D-2-d-(1). Sewer vents and other ventilation lines contain high efficiency particulate air/HEPA filters. HEPA filters shall be certified annually.

Appendix P-II-D-2-d-(2). A pass-through dunk tank, fumigation chamber, or an equivalent method of decontamination shall be provided to ensure decontamination of materials and equipment that cannot be decontaminated in the autoclave.

Appendix P-II-D-2-d-(3). Liquid effluent from sinks, floors, and autoclave chambers shall be decontaminated by heat or chemical treatment before being released from the maximum containment greenhouse facility. Liquid wastes from shower rooms and toilets may be decontaminated by heat or chemical treatment. Autoclave and chemical decontamination of liquid wastes shall be evaluated by appropriate standard procedures for autoclaved wastes. Decontamination shall be evaluated mechanically and biologically using a recording thermometer and an indicator microorganism with a defined heat susceptibility pattern. If liquid wastes are decontaminated with chemical disinfectants, the chemicals used must have demonstrated efficacy against the target or indicator microorganisms.

Appendix P-II-D-2-d-(4). If there is a central vacuum system, it shall not serve areas outside of the greenhouse facility. In-line high efficiency particulate air/HEPA filters shall be placed as near as practicable to each use point or vacuum service cock. Other liquid and gas services to the greenhouse facility shall be protected by devices that prevent back-flow. HEPA filters shall be certified annually.

Appendix P-III. Biological Containment Practices

Appropriate selection of the following biological containment practices may be used to meet the containment requirements for a given organism. The present list is not exhaustive; there may be other ways of preventing effective dissemination that could possibly lead to the establishment of the organism or its genetic material in the environment resulting in deleterious consequences to managed or natural ecosystems.

Appendix P-III-A. Biological Containment Practices (Plants)

Appendix P-III-A-1. Effective dissemination of plants by pollen or seed can be prevented by one or more of the following procedures: (i) cover the reproductive structures to prevent pollen dissemination at flowering and seed dissemination at maturity; (ii) remove reproductive structures by employing male sterile strains, or harvest the plant material prior to the reproductive stage; (iii) ensure that experimental plants flower at a time of year when cross-fertile plants are not flowering within the normal pollen dispersal range of the experimental plant; or (iv) ensure that cross-fertile plants are not growing within the known pollen dispersal range of the experimental plant.

Appendix P-III-B. Biological Containment Practices (Microorganisms)

Appendix P-III-B-1. Effective dissemination of microorganisms beyond the confines of the greenhouse can be prevented by one or more of the following procedures: (i) confine all operations to injections of microorganisms or other biological procedures (including genetic manipulation) that limit replication or reproduction of viruses and microorganisms or sequences derived from microorganisms, and confine these injections to internal plant parts or adherent plant surfaces; (ii) ensure that organisms, which can serve as hosts or promote the transmission of the virus or microorganism, are not present within the farthest distance that the airborne virus or microorganism may be expected to be effectively disseminated; (iii) conduct experiments at a time of year when plants that can serve as hosts are either not growing or are not susceptible to productive infection; (iv) use viruses and other microorganisms or their genomes that have known arthropod or animal vectors, in the absence of such vectors; (v) use microorganisms that have an obligate association with the plant; or (vi) use microorganisms that are genetically disabled to minimize survival outside of the research facility and whose natural mode of transmission requires injury of the target organism, or assures that inadvertent release is unlikely to initiate productive infection of organisms outside of the experimental facility.

Appendix P-III-C. Biological Containment Practices (Macroorganisms)

Appendix P-III-C-1. Effective dissemination of arthropods and other small animals can be prevented by using one or more of the following procedures: (i) use non-flying, flight-impaired, or sterile arthropods; (ii) use non-motile or sterile strains of small animals; (iii) conduct experiments at a time of year that precludes the survival of escaping organisms; (iv) use animals that have an obligate association with a plant that is not present within the dispersal range of the organism; or (v) prevent the escape of organisms present in run-off water by chemical treatment or evaporation of run-off water.

APPENDIX Q. PHYSICAL AND BIOLOGICAL CONTAINMENT FOR RECOMBINANT DNA RESEARCH INVOLVING ANIMALS

Appendix Q specifies containment and confinement practices for research involving whole animals, both those in which the animal's genome has been altered by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant DNA-modified microorganisms tested on whole animals. The appendix applies to animal research activities with the following modifications:

Appendix Q shall supersede [Appendix G \(Physical Containment\)](#) when research animals are of a size or have growth requirements that preclude the use of containment for laboratory animals. Some animals may require other types of containment (see [Appendix Q-III-D, Footnotes and References for Appendix Q](#)). The animals covered in Appendix Q are those species normally categorized as animals including but not limited to cattle, swine, sheep, goats, horses, and poultry.

The Institutional Biosafety Committee shall include at least one scientist with expertise in animal containment principles when experiments utilizing Appendix Q require Institutional Biosafety Committee prior approval.

The institution shall establish and maintain a health surveillance program for personnel engaged in animal research involving viable recombinant DNA-containing microorganisms that require Biosafety Level (BL) 3 or greater containment in the laboratory.

Appendix Q-I. General Considerations

Appendix Q-I-A. Containment Levels

The containment levels required for research involving recombinant DNA associated with or in animals is based on classification of experiments in [Section III, Experiments Covered by the NIH Guidelines](#). For the purpose of animal research, four levels of containment are established. These are referred to as BL1-Animals (N), BL2-N, BL3-N, and BL4-N and are described in the following appendices of Appendix Q. The descriptions include: (i) standard practices for physical and biological containment, and (ii) animal facilities.

Appendix Q-I-B. Disposal of Animals (BL1-N through BL4-N)

Appendix Q-I-B-1. When an animal covered by Appendix Q containing recombinant DNA or a recombinant DNA-derived organism is euthanized or dies, the carcass shall be disposed of to avoid its use as food for human beings or animals unless food use is specifically authorized by an appropriate Federal agency.

Appendix Q-I-B-2. A permanent record shall be maintained of the experimental use and disposal of each animal or group of animals.

Appendix Q-II. Physical and Biological Containment Levels**Appendix Q-II-A. Biosafety Level 1 - Animals (BL1-N)****Appendix Q-II-A-1. Standard Practices (BL1-N)****Appendix Q-II-A-1-a. Animal Facility Access (BL1-N)**

Appendix Q-II-A-1-a-(1). The containment area shall be locked.

Appendix Q-II-A-1-a-(2). Access to the containment area shall be limited or restricted when experimental animals are being held.

Appendix Q-II-A-1-a-(3). The containment area shall be patrolled or monitored at frequent intervals.

Appendix Q-II-A-1-b. Other (BL1-N)

Appendix Q-II-A-1-b-(1). All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

Appendix Q-II-A-1-b-(2) A double barrier shall be provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used.

Appendix Q-II-A-1-b-(3). The containment area shall be in accordance with state and Federal laws and animal care requirements.

Appendix Q-II-A-2. Animal Facilities (BL1-N)

Appendix Q-II-A-2-a. Animals shall be confined to securely fenced areas or be in enclosed structures (animal rooms) to minimize the possibility of theft or unintentional release.

Appendix Q-II-B. Biosafety Level 2 - Animals (BL2-N) (See [Appendix Q-III-A, Footnotes and References for Appendix Q](#))

Appendix Q-II-B-1. Standard Practices (BL2-N)**Appendix Q-II-B-1-a. Animal Facility Access (BL2-N)**

Appendix Q-II-B-1-a-(1). The containment area shall be locked.

Appendix Q-II-B-1-a-(2). The containment area shall be patrolled or monitored at frequent intervals.

Appendix Q-II-B-1-a-(3). The containment building shall be controlled and have a locking access.

Appendix Q-II-B-1-a-(4). The Animal Facility Director shall establish policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) may enter the laboratory or animal rooms.

Appendix Q-II-B-1-a-(5). Animals of the same or different species, which are not involved in the work being performed, shall not be permitted in the animal area.

Appendix Q-II-B-1-b. Decontamination and Inactivation (BL2-N)

Appendix Q-II-B-1-b-(1). Contaminated materials that are decontaminated at a site away from the laboratory shall be placed in a closed durable leak-proof container prior to removal from the laboratory.

Appendix Q-II-B-1-b-(2). Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q-II-B-1-c. Signs (BL2-N)

Appendix Q-II-B-1-c-(1). When the animal research requires special provisions for entry (e.g., vaccination), a warning sign incorporating the universal biosafety symbol shall be posted on all access doors to the animal work area. The sign shall indicate: (i) the agent, (ii) the animal species, (iii) the name and telephone number of the Animal Facility Director or other responsible individual, and (iv) any special requirements for entering the laboratory.

Appendix Q-II-B-1-d. Protective Clothing (BL2-N)

Appendix Q-II-B-1-d-(1). Laboratory coats, gowns, smocks, or uniforms shall be worn while in the animal area or attached laboratory. Before entering non-laboratory areas (e.g., cafeteria, library, administrative offices), protective clothing shall be removed and kept in the work entrance area.

Appendix Q-II-B-1-d-(2). Special care shall be taken to avoid skin contamination with microorganisms containing recombinant DNA. Impervious and/or protective gloves shall be worn when handling experimental animals and when skin contact with an infectious agent is unavoidable.

Appendix Q-II-B-1-e. Records (BL2-N)

Appendix Q-II-B-1-e-(1). Any incident involving spills and accidents that result in environmental release or exposures of animals or laboratory workers to organisms containing recombinant DNA molecules shall be reported immediately to the Animal Facility Director, Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities (if applicable). Reports to the NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.

Appendix Q-II-B-1-e-(2). When appropriate and giving consideration to the agent handled, baseline serum samples shall be collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agent handled and the function of the animal facility.

Appendix Q-II-B-1-f. Transfer of Materials (BL2-N)

Appendix Q-II-B-1-f-(1). Biological materials removed from the animal containment area in a viable or intact state shall be transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container. All containers, primary and secondary, shall be disinfected before removal from the animal facility. Advance approval for transfer of material shall be obtained from the Animal Facility Director. Packages containing viable agents may only be opened in a facility having an equivalent or higher level of physical containment unless the agent is biologically inactivated or incapable of reproduction.

Appendix Q-II-B-1-g. Other (BL2-N)

Appendix Q-II-B-1-g-(1). All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

Appendix Q-II-B-1-g-(2). Needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) shall be used for the injection or aspiration of fluids containing organisms that contain recombinant DNA. Extreme caution shall be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Following use, needles shall not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe. Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q-II-B-1-g-(3). Appropriate steps should be taken to prevent horizontal transmission or exposure of laboratory personnel. If the agent used as a vector is known to be transmitted by a particular route (e.g., arthropods), special attention should be given to preventing spread by that route. In the absence of specific knowledge of a particular route of transmission, all potential means of horizontal transmission (e.g., arthropods, contaminated bedding, or animal waste, etc.) should be prevented.

Appendix Q-II-B-1-g-(4). Eating, drinking, smoking, and applying cosmetics shall not be permitted in the work area.

Appendix Q-II-B-1-g-(5). Individuals who handle materials and animals containing recombinant DNA molecules shall be required to wash their hands before exiting the containment area.

Appendix Q-II-B-1-g-(6). A double barrier shall be provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used.

Appendix Q-II-B-1-g-(7). The containment area shall be in accordance with state and Federal laws and animal care requirements.

Appendix Q-II-B-1-g-(8). A biosafety manual shall be prepared or adopted. Personnel shall be advised of special hazards and required to read and follow instructions on practices and procedures.

Appendix Q-II-B-2. Animal Facilities (BL2-N)

Appendix Q-II-B-2-a. Animals shall be contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and to avoid arthropod access. The special provision to avoid the entry or escape of arthropods from the animal areas may be waived if the agent in use is not known to be transmitted by arthropods.

Appendix Q-II-B-2-b. Surfaces shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix Q-II-B-2-c. The animal containment area shall be designed so that it can be easily cleaned.

Appendix Q-II-B-2-d. Windows that open shall be fitted with fly screens.

Appendix Q-II-B-2-e. An autoclave shall be available for decontamination of laboratory wastes.

Appendix Q-II-B-2-f. If arthropods are used in the experiment or the agent under study can be transmitted by an arthropod, interior work areas shall be appropriately screened (52 mesh). All perimeter joints and openings shall be sealed and additional arthropod control mechanisms used to minimize arthropod entry and propagation, including appropriate screening of access doors or the equivalent.

Appendix Q-II-C. Biosafety Level 3 - Animals (BL3-N) (See [Appendix Q-III-B, Footnotes and References for Appendix Q](#))

Appendix Q-II-C-1. Standard Practices (BL3-N)

Appendix Q-II-C-1-a. Animal Facility Access (BL3-N)

Appendix Q-II-C-1-a-(1). The containment area shall be locked.

Appendix Q-II-C-1-a-(2). The containment area shall be patrolled or monitored at frequent intervals.

Appendix Q-II-C-1-a-(3). The containment building shall be controlled and have a locking access.

Appendix Q-II-C-1-a-(4). The Animal Facility Director shall establish policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) shall enter the laboratory or animal rooms.

Appendix Q-II-C-1-a-(5). Animal room doors, gates, or other closures shall be kept closed when experiments are in progress.

Appendix Q-II-C-1-b. Decontamination and Inactivation (BL3-N)

Appendix Q-II-C-1-b-(1). The work surfaces of containment equipment shall be decontaminated when work with organisms containing recombinant DNA molecules is finished. Where feasible, plastic-backed paper toweling shall be used on nonporous work surfaces to facilitate clean-up.

Appendix Q-II-C-1-b-(2). All animals shall be euthanized at the end of their experimental usefulness and the carcasses decontaminated before disposal in an approved manner.

Appendix Q-II-C-1-b-(3). Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q-II-C-1-b-(4). Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval shall be required to transfer agents or tissue/organ specimens from a BL3-N animal facility to a facility with a lower containment classification.

Appendix Q-II-C-1-b-(5). Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated every 30 days with an indicator organism.

Appendix Q-II-C-1-c. Signs (BL3-N)

Appendix Q-II-C-1-c-(1). When the animal research requires special provisions for entry (e.g., vaccination), a warning sign incorporating the universal biosafety symbol shall be posted on all access doors to the animal work area. The sign shall indicate: (i) the agent, (ii) the animal species, (iii) the name and telephone number of the Animal Facility Director or other responsible individual, and (iv) any special requirements for entering the laboratory.

Appendix Q-II-C-1-d. Protective Clothing (BL3-N)

Appendix Q-II-C-1-d-(1). Full protective clothing that protects the individual (e.g., scrub suits, coveralls, uniforms) shall be worn in the animal area. Clothing shall not be worn outside the animal containment area and shall be decontaminated before laundering or disposal. Personnel shall be required to shower before exiting the BL3-N area and wearing of personal clothing.

Appendix Q-II-C-1-d-(2). Special care shall be taken to avoid skin contamination with microorganisms containing recombinant DNA. Impervious and/or protective gloves shall be worn when handling experimental animals and when skin contact with an infectious agent is unavoidable.

Appendix Q-II-C-1-d-(3). Appropriate respiratory protection shall be worn in rooms containing experimental animals.

Appendix Q-II-C-1-e. Records (BL3-N)

Appendix Q-II-C-1-e-(1). Documents regarding experimental animal use and disposal shall be maintained in a permanent record book.

Appendix Q-II-C-1-e-(2). Any incident involving spills and accidents that result in environmental release or exposure of animals or laboratory workers to organisms containing recombinant DNA shall be reported immediately to the Biological Safety Office, Animal Facility Director, Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities (if applicable). Reports to the NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.

Appendix Q-II-C-1-e-(3). When appropriate and giving consideration to the agent handled, baseline serum samples shall be collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agent handled or the function of the facility.

Appendix Q-II-C-1-f. Transfer of Materials (BL3-N)

Appendix Q-II-C-1-f-(1). Biological materials removed from the animal containment laboratory in a viable or intact state shall be transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container. All containers, primary and secondary, shall be disinfected before removal from the animal facility. Advance approval for transfer of material shall be obtained from the Animal Facility Director. Packages containing viable agents may be opened only in a facility having an equivalent or higher level of physical containment unless the agent is biologically inactivated or incapable of reproduction.

Appendix Q-II-C-1-f-(2). Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval shall be required to transfer agents or tissue/organ specimens from a BL3-N animal facility to a facility with a lower containment classification.

Appendix Q-II-C-1-g. Other (BL3-N)

Appendix Q-II-C-1-g-(1). All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

Appendix Q-II-C-1-g-(2). Appropriate steps should be taken to prevent horizontal transmission or exposure of laboratory personnel. If the agent used as the vector is known to be transmitted by a particular route (e.g., arthropods), special attention should be given to preventing spread by that route. In the absence of specific knowledge of a particular route of transmission, all potential means of horizontal transmission (e.g., arthropods, contaminated bedding, or animal waste) should be prevented.

Appendix Q-II-C-1-g-(3). Eating, drinking, smoking, and applying cosmetics shall not be permitted in the work area.

Appendix Q-II-C-1-g-(4). Individuals who handle materials and animals containing recombinant DNA molecules shall be required to wash their hands before exiting the containment area.

Appendix Q-II-C-1-g-(5). Experiments involving other organisms that require containment levels lower than BL3-N may be conducted in the same area concurrently with experiments requiring BL3-N containment provided that they are conducted in accordance with BL3-N practices.

Appendix Q-II-C-1-g-(6). Animal holding areas shall be cleaned at least once a day and decontaminated immediately following any spill of viable materials.

Appendix Q-II-C-1-g-(7). All procedures shall be performed carefully to minimize the creation of aerosols.

Appendix Q-II-C-1-g-(8). A double barrier shall be provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used.

Appendix Q-II-C-1-g-(9). The containment area shall be in accordance with state and Federal laws and animal care requirements.

Appendix Q-II-C-1-g-(10). All animals shall be euthanized at the end of their experimental usefulness and the carcasses decontaminated before disposal in an approved manner.

Appendix Q-II-C-1-g-(11). Personnel shall be required to shower before exiting the BL3-N area and wearing personal clothing.

Appendix Q-II-C-1-g-(12). Animals of the same or different species, which are not involved in the work being performed, shall not be permitted in the animal area.

Appendix Q-II-C-1-g-(13). Needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) shall be used for the injection or aspiration of fluids containing organisms that contain recombinant DNA. Extreme caution shall be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Following use, needles shall not be bent, sheared, replaced in the needle sheath or guard or removed from the syringe. The needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q-II-C-1-g-(14). A biosafety manual shall be prepared or adopted. Personnel shall be advised of special hazards and required to read and follow instructions on practices and procedures.

Appendix Q-II-C-2. Animal Facilities (BL3-N)

Appendix Q-II-C-2-a. Animals shall be contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and avoid arthropod access. The special provision to avoid the entry or escape of arthropods from the animal areas may be waived if the agent in use is not known to be transmitted by arthropods.

Appendix Q-II-C-2-b. The interior walls, floors, and ceilings shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat, to facilitate cleaning. Penetrations in these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

Appendix Q-II-C-2-c. Windows in the animal facility shall be closed, sealed, and breakage resistant (e.g., double-pane tempered glass or equivalent). The need to maintain negative pressure should be considered when constructing or renovating the animal facility.

Appendix Q-II-C-2-d. An autoclave, incinerator, or other effective means to decontaminate animals and waste shall be available, preferably within the containment area. If feasible, a double-door autoclave is preferred and should be positioned to allow removal of material from the containment area.

Appendix Q-II-C-2-e. If arthropods are used in the experiment or the agent under study can be transmitted by an arthropod, the interior work area shall be appropriately screened (52 mesh). All perimeter joints and openings shall be sealed, and additional arthropod control mechanisms used to minimize arthropod entry and propagation, including appropriate screening, or the equivalent of access doors.

Appendix Q-II-C-2-f. Access doors to the containment area shall be self-closing.

Appendix Q-II-C-2-g. The animal area shall be separated from all other areas. Passage through two sets of doors shall be the basic requirement for entry into the animal area from access corridors or other contiguous areas. The animal containment area shall be physically separated from access corridors and other laboratories or areas by a double-door clothes change room, equipped with integral showers and airlock.

Appendix Q-II-C-2-h. Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated every 30 days with an indicator organism.

Appendix Q-II-C-2-i. An exhaust air ventilation system shall be provided. This system shall create directional airflow that draws air into the animal room through the entry area. The building exhaust, or the exhaust from primary containment units, may be used for this purpose if the exhaust air is discharged to the outside and shall be dispersed away from occupied areas and air intakes. Personnel shall verify that the direction of the airflow (into the animal room) is proper.

Appendix Q-II-C-2-j. If the agent is transmitted by aerosol, then the exhaust air shall pass through a high efficiency particulate air/HEPA filter.

Appendix Q-II-C-2-k. Vacuum lines shall be protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.

Appendix Q-II-C-2-l. In lieu of open housing in the special animal room, animals held in a BL3-N area may be housed in partial-containment caging systems (e.g., Horsfall units or gnotobiotic systems, or other special containment primary barriers). Prudent judgment must be exercised to implement this ventilation system (e.g., animal species) and its discharge location.

Appendix Q-II-C-2-m. Each animal area shall contain a foot, elbow, or automatically operated sink for hand washing. The sink shall be located near the exit door.

Appendix Q-II-C-2-n. Restraining devices for animals may be required to avoid damage to the integrity of the animal containment facility.

Appendix Q-II-D. Biosafety Level 4 - Animals (BL4-N) (See [Appendix Q-III-C](#), *Footnotes and References for Appendix Q*)

Appendix Q-II-D-1. Standard Practices (BL4-N)

Appendix Q-II-D-1-a. Animal Facility Access (BL4-N)

Appendix Q-II-D-1-a-(1). Individuals under 16 years of age shall not be permitted to enter the animal area.

Appendix Q-II-D-1-a-(2). The containment area shall be locked.

Appendix Q-II-D-1-a-(3). The containment area shall be patrolled or monitored at frequent intervals.

Appendix Q-II-D-1-a-(4). The containment building shall be controlled and have a locking access.

Appendix Q-II-D-1-a-(5). The Animal Facility Director shall establish policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) may enter the laboratory or animal room.

Appendix Q-II-D-1-a-(6). Individuals shall enter and exit the animal facility only through the clothing change and shower rooms.

Appendix Q-II-D-1-a-(7). Personnel shall use the airlocks to enter or exit the laboratory only in an emergency.

Appendix Q-II-D-1-a-(8). Animal room doors, gates, and other closures shall be kept closed when experiments are in progress.

Appendix Q-II-D-1-b. Decontamination and Inactivation (BL4-N)

Appendix Q-II-D-1-b-(1). All contaminated liquid or solid wastes shall be decontaminated before disposal.

Appendix Q-II-D-1-b-(2). The work surfaces and containment equipment shall be decontaminated when work with organisms containing recombinant DNA molecules is finished. Where feasible, plastic-backed paper toweling shall be used on nonporous work surfaces to facilitate clean-up.

Appendix Q-II-D-1-b-(3). All wastes from animal rooms and laboratories shall be appropriately decontaminated before disposal in an approved manner.

Appendix Q-II-D-1-b-(4). No materials, except for biological materials that are to remain in a viable or intact state, shall be removed from the maximum containment laboratory unless they have been autoclaved or decontaminated. Equipment or material that might be damaged by high temperatures or steam shall be decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

Appendix Q-II-D-1-b-(5). When ventilated suits are required, the animal personnel shower entrance/exit area shall be equipped with a chemical disinfectant shower to decontaminate the surface of the suit before exiting the area. A neutralization or water dilution device shall be integral with the chemical disinfectant discharge piping before entering the heat sterilization system. Entry to this area shall be through an airlock fitted with airtight doors.

Appendix Q-II-D-1-b-(6). Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q-II-D-1-b-(7). Supplies and materials needed in the animal facility shall be brought in by way of the double-door autoclave, fumigation chamber, or airlock that shall be appropriately decontaminated between each use.

Appendix Q-II-D-1-b-(8). An autoclave, incinerator, or other effective means to decontaminate animals and wastes shall be available, preferably within the containment area. If feasible, a double-door autoclave is preferred and should be positioned to allow removal of material from the containment area.

Appendix Q-II-D-1-b-(9). Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. Liquid wastes from shower rooms and toilets shall be decontaminated with chemical disinfectants or heat by methods demonstrated to be effective. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated every 30 days with an indicator organism. Liquid wastes from the shower shall be chemically decontaminated using an Environmental Protection Agency-approved germicide. The efficacy of the chemical treatment process shall be validated with an indicator organism. Chemical disinfectants shall be neutralized or diluted before release into general effluent waste systems.

Appendix Q-II-D-1-c. Signs (BL4-N)

Appendix Q-II-D-1-c-(1). When the animal research requires special provisions for entry (e.g., vaccination), a warning sign incorporating the universal biosafety symbol shall be posted on all access doors to the animal work area. The sign shall indicate: (i) the agent, (ii) the animal species, (iii) the name and telephone number of the Animal Facility Director, or other responsible individual, and (iv) any special requirements for entering the laboratory.

Appendix Q-II-D-1-d. Protective Clothing (BL4-N)

Appendix Q-II-D-1-d-(1). Individuals shall enter and exit the animal facility only through the clothing change and shower rooms. Street clothing shall be removed and kept in the outer clothing change room. Complete laboratory clothing (may be disposable), including undergarments, pants, shirts, jump suits, and shoes shall be provided for all personnel entering the animal facility. When exiting the BL4-N area and before proceeding into the shower area, personnel shall remove their laboratory clothing in the inner change room. All laboratory clothing shall be autoclaved before laundering. Personnel shall shower each time they exit the animal facility.

Appendix Q-II-D-1-d-(2). A ventilated head-hood or a one-piece positive pressure suit, which is ventilated by a life-support system, shall be worn by all personnel entering rooms that contain experimental animals when appropriate. When ventilated suits are required, the animal personnel shower entrance/exit area shall be equipped with a chemical disinfectant shower to decontaminate the surface of the suit before exiting the area. A neutralization or water dilution device shall be integral with the chemical disinfectant discharge piping before entering the heat sterilization system. Entry to this area shall be through an airlock fitted with airtight doors.

Appendix Q-II-D-1-d-(3). Appropriate respiratory protection shall be worn in rooms containing experimental animals.

Appendix Q-II-D-1-e. Records (BL4-N)

Appendix Q-II-D-1-e-(1). Documents regarding experimental animal use and disposal shall be maintained in a permanent record book.

Appendix Q-II-D-1-e-(2). A system shall be established for: (i) reporting laboratory accidents and exposures that are a result of overt exposures to organisms containing recombinant DNA, (ii) employee absenteeism, and (iii) medical surveillance of potential laboratory-associated illnesses. Permanent records shall be prepared and maintained. Any incident involving spills and accidents that results in environmental release or exposures of animals or laboratory workers to organisms containing recombinant DNA molecules shall be reported immediately to the Biological Safety Officer, Animal Facility Director, Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities (if applicable). Reports to the NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.

Appendix Q-II-D-1-e-(3). When appropriate and giving consideration to the agents handled, baseline serum samples shall be collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agents handled or the function of the facility.

Appendix Q-II-D-1-e-(4). A permanent record book indicating the date and time of each entry and exit shall be signed by all personnel.

Appendix Q-II-D-1-f. Transfer of Materials (BL4-N)

Appendix Q-II-D-1-f-(1). No materials, except for biological materials that are to remain in a viable or intact state, shall be removed from the maximum containment laboratory unless they have been autoclaved or decontaminated. Equipment or material that might be damaged by high temperatures or steam shall be decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

Appendix Q-II-D-1-f-(2). Biological materials removed from the animal maximum containment laboratory in a viable or intact state shall be transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container that shall be removed from the animal facility through a disinfectant dunk tank, fumigation chamber, or an airlock designed for this purpose. Advance approval for transfer of material shall be obtained from the Animal Facility Director. Such packages containing viable agents can only be opened in another BL4-N animal facility if the agent is biologically inactivated or incapable of reproduction. Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval shall be required to transfer agents or tissue/organ specimens from a BL4-N animal facility to one with a lower containment classification.

Appendix Q-II-D-1-f-(3). Supplies and materials needed in the animal facility shall be brought in by way of the double-door autoclave, fumigation chamber, or airlock that shall be appropriately decontaminated between each use. After securing the outer doors, personnel within the animal facility retrieve the materials by opening the interior doors of the autoclave, fumigation chamber, or airlock. These doors shall be secured after materials are brought into the animal facility.

Appendix Q-II-D-1-g. Other (BL4-N)

Appendix Q-II-D-1-g-(1). All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

Appendix Q-II-D-1-g-(2). Eating, drinking, smoking, and applying cosmetics shall not be permitted in the work area.

Appendix Q-II-D-1-g-(3). Individuals who handle materials and animals containing recombinant DNA molecules shall be required to wash their hands before exiting the containment area.

Appendix Q-II-D-1-g-(4). Experiments involving other organisms that require containment levels lower than BL4-N may be conducted in the same area concurrently with experiments requiring BL4-N containment provided that they are conducted in accordance with BL4-N practices.

Appendix Q-II-D-1-g-(5). Animal holding areas shall be cleaned at least once a day and decontaminated immediately following any spill of viable materials.

Appendix Q-II-D-1-g-(6). All procedures shall be performed carefully to minimize the creation of aerosols.

Appendix Q-II-D-1-g-(7). A double barrier shall be provided to separate male and female animals. Animal isolation barriers shall be sturdy and accessible for cleaning. Reproductive incapacitation may be used.

Appendix Q-II-D-1-g-(8). The containment area shall be in accordance with state and Federal laws and animal care requirements.

Appendix Q-II-D-1-g-(9). The life support system for the ventilated suit or head hood is equipped with alarms and emergency back-up air tanks. The exhaust air from the suit area shall be filtered by two sets of high efficiency particulate air/HEPA filters installed in series or incinerated. A duplicate filtration unit, exhaust fan, and an automatically starting emergency power source shall be provided. The air pressure within the suit shall be greater than that of any adjacent area. Emergency lighting and communication systems shall be provided. A double-door autoclave shall be provided for decontamination of waste materials to be removed from the suit area.

Appendix Q-II-D-1-g-(10). Needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) shall be used for the injection or aspiration of fluids containing organisms that contain recombinant DNA. Extreme caution shall be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Following use, needles shall

not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe. The needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q-II-D-1-g-(11). An essential adjunct to the reporting-surveillance system is the availability of a facility for quarantine, isolation, and medical care of personnel with potential or known laboratory-associated illnesses.

Appendix Q-II-D-1-g-(12). A biosafety manual shall be prepared or adopted. Personnel shall be advised of special hazards and required to read and follow instructions on practices and procedures.

Appendix Q-II-D-1-g-(13). Vacuum lines shall be protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.

Appendix Q-II-D-2. Animal Facilities (BL4-N)

Appendix Q-II-D-2-a. Animals shall be contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and avoid arthropod access.

Appendix Q-II-D-2-b. The interior walls, floors, and ceilings shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat, to facilitate cleaning. Penetrations in these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

Appendix Q-II-D-2-c. Windows in the animal facility shall be closed, sealed, and breakage resistant (e.g., double-pane tempered glass or equivalent).

Appendix Q-II-D-2-d. An autoclave, incinerator, or other effective means to decontaminate animals and wastes shall be available, preferably within the containment area. If feasible, a double-door autoclave is preferred and should be positioned to allow removal of material from the containment area.

Appendix Q-II-D-2-e. Access doors to the containment area shall be self-closing.

Appendix Q-II-D-2-f. All perimeter joints and openings shall be sealed to form an arthropod-proof structure.

Appendix Q-II-D-2-g. The BL4-N laboratory provides a double barrier to prevent the release of recombinant DNA containing microorganisms into the environment. Design of the animal facility shall be such that if the barrier of the inner facility is breached, the outer barrier will prevent release into the environment. The animal area shall be separated from all other areas. Passage through two sets of doors shall be the basic requirement for entry into the animal area from access corridors or other contiguous areas. Physical separation of the animal containment area from access corridors or other laboratories or activities shall be provided by a double-door clothes change room equipped with integral showers and airlock.

Appendix Q-II-D-2-h. A necropsy room shall be provided within the BL4-N containment area.

Appendix Q-II-D-2-i. Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. Liquid wastes from shower rooms and toilets shall be decontaminated with chemical disinfectants or heat by methods demonstrated to be effective. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated every 30 days with an indicator organism. Liquid wastes from the shower shall be chemically decontaminated using an Environmental Protection Agency-approved germicide. The efficacy of the chemical treatment process shall be validated with an indicator organism. Chemical disinfectants shall be neutralized or diluted before release into general effluent waste systems.

Appendix Q-II-D-2-j. A ducted exhaust air ventilation system shall be provided that creates directional airflow that draws air into the laboratory through the entry area. The exhaust air, which is not recirculated to any other area of the building, shall be discharged to the outside and dispersed away from the occupied areas and air intakes. Personnel shall verify that the direction of the airflow (into the animal room) is proper.

Appendix Q-II-D-2-k. Exhaust air from BL4-N containment area shall be double high efficiency particulate air/HEPA filtered or treated by passing through a certified HEPA filter and an air incinerator before release to the atmosphere. Double HEPA filters shall be required for the supply air system in a BL4-N containment area.

Appendix Q-II-D-2-l. All high efficiency particulate air/HEPA filters' frames and housings shall be certified to have no detectable smoke [dioctylphthalate] leaks when the exit face (direction of flow) of the filter is scanned above 0.01 percent when measured by a linear or logarithmic photometer. The instrument must demonstrate a threshold sensitivity of at least 1×10^{-3} micrograms per liter for 0.3 micrometer diameter dioctylphthalate particles and a challenge concentration of 80-120 micrograms per liter. The air sampling rate should be at least 1 cfm (28.3 liters per minute).

Appendix Q-II-D-2-m. If an air incinerator is used in lieu of the second high efficiency particulate air/HEPA filter, it shall be biologically challenged to prove all viable test agents are sterilized. The biological challenge must be minimally 1×10^8 organisms per cubic foot of airflow through the incinerator. It is universally accepted if bacterial spores are used to challenge and verify that the equipment is capable of killing spores, then assurance is provided that all other known agents are inactivated by the parameters established to operate the equipment. Test spores meeting this criterion are *Bacillus subtilis* var. *niger* or *Bacillus stearothermophilis*. The operating temperature of the incinerator shall be continuously monitored and recorded during use.

Appendix Q-II-D-2-n. All equipment and floor drains shall be equipped with deep traps (minimally 5 inches). Floor drains shall be fitted with isolation plugs or fitted with automatic water fill devices.

Appendix Q-II-D-2-o. Each animal area shall contain a foot, elbow, or automatically operated sink for hand washing. The sink shall be located near the exit door.

Appendix Q-II-D-2-p. Restraining devices for animals may be required to avoid damage to the integrity of the containment animal facility.

Appendix Q-II-D-2-q. The supply water distribution system shall be fitted with a back-flow preventer or break tank.

Appendix Q-II-D-2-r. All utilities, liquid and gas services, shall be protected with devices that avoid back-flow.

Appendix Q-II-D-2-s. Sewer and other atmospheric ventilation lines shall be equipped minimally with a single high efficiency particulate/HEPA filter. Condensate drains from these type housings shall be appropriately connected to a contaminated or sanitary drain system. The drain position in the housing dictates the appropriate system to be used.

Appendix Q-III. Footnotes and References for Appendix Q

Appendix Q-III-A. If recombinant DNA is derived from a Class 2 organism requiring BL2 containment, personnel shall be required to have specific training in handling pathogenic agents and directed by knowledgeable scientists.

Appendix Q-III-B. Personnel who handle pathogenic and potentially lethal agents shall be required to have specific training and be supervised by knowledgeable scientists who are experienced in working with these agents. BL3-N containment also minimizes escape of recombinant DNA-containing organisms from exhaust air or waste material from the containment area.

Appendix Q-III-C. Risk Group 4 and restricted microorganisms (see [Appendix B, Classification of Human Etiologic Agents on the Basis of Hazard](#), and [Sections V-G and V-L, Footnotes and References of Sections I through IV](#)) pose a high level of individual risk for acquiring life-threatening diseases to personnel and/or animals. To import animal or plant pathogens, special approval must be obtained from [U.S. Department of Agriculture, Animal and Plant Health Inspection Service \(APHIS\)](#), Veterinary Services, National Center for Import-Export, Products Program, 4700 River Road, Unit 40, Riverdale, MD 20737. Phone: (301) 734-8499; Fax: (301) 734-8226.

Laboratory staff shall be required to have specific and thorough training in handling extremely hazardous infectious agents, primary and secondary containment, standard and special practices, and laboratory design characteristics. The laboratory staff shall be supervised by knowledgeable scientists who are trained and experienced in working with these agents and in the special containment facilities.

Within work areas of the animal facility, all activities shall be confined to the specially equipped animal rooms or support areas. The maximum animal containment area and support areas shall have special engineering and design features to prevent the dissemination of microorganisms into the environment via exhaust air or waste disposal.

Appendix Q-III-D. Other research with non-laboratory animals, which may not appropriately be conducted under conditions described in [Appendix Q](#), may be conducted safely by applying practices routinely used for controlled culture of these biota. In aquatic systems, for example, BL1 equivalent conditions could be met by utilizing growth tanks that provide adequate physical means to avoid the escape of the aquatic species, its gametes, and introduced exogenous genetic material. A mechanism shall be provided to ensure that neither the organisms nor their gametes can escape into the supply or discharge system of the rearing container (e.g., tank, aquarium, etc.) Acceptable barriers include appropriate filtration, irradiation, heat treatment, chemical treatment, etc. Moreover, the top of the rearing container shall be covered to avoid escape of the organism and its gametes. In the event of tank rupture, leakage, or overflow, the construction of the room containing these tanks should prevent the organisms and gametes from entering the building's drains before the organism and its gametes have been inactivated.

Other types of non-laboratory animals (e.g., nematodes, arthropods, and certain forms of smaller animals) may be accommodated by using the appropriate BL1 through BL4 or BL1-P through BL4-P containment practices and procedures as specified in [Appendices G and P](#).

Biosafety in Microbiological and Biomedical Laboratories

5th Edition



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National Institutes of Health

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Foreword

Biosafety in Microbiological and Biomedical Laboratories (BMBL) quickly became the cornerstone of biosafety practice and policy in the United States upon first publication in 1984. Historically, the information in this publication has been advisory in nature even though legislation and regulation, in some circumstances, have overtaken it and made compliance with the guidance provided mandatory. We wish to emphasize that the 5th edition of the BMBL remains an advisory document recommending best practices for the safe conduct of work in biomedical and clinical laboratories from a biosafety perspective, and is not intended as a regulatory document though we recognize that it will be used that way by some.

This edition of the BMBL includes additional sections, expanded sections on the principles and practices of biosafety and risk assessment; and revised agent summary statements and appendices. We worked to harmonize the recommendations included in this edition with guidance issued and regulations promulgated by other federal agencies. Wherever possible, we clarified both the language and intent of the information provided. The events of September 11, 2001, and the anthrax attacks in October of that year re-shaped and changed, forever, the way we manage and conduct work in biological and clinical laboratories and drew into focus the need for inclusion of additional information in the BMBL. To better serve the needs of our community in this new era, this edition includes information on the following topics:

- Occupational medicine and immunization
- Decontamination and sterilization
- Laboratory biosecurity and risk assessment
- Biosafety level 3 (Ag) laboratories
- Agent summary statements for some agricultural pathogens
- Biological toxins

At last count, over two hundred of our scientific and professional colleagues have assisted in the preparation of the 5th edition through participation in technical working groups, serving as reviewers and guest editors, and as subject matter experts. We wish to thank them all for their dedication and hard work for without them the 5th edition of the BMBL would not be possible. We also recognize the hard work and contributions made by all who participated in preparation of the previous editions of the BMBL; we have built on their solid work and commitment. It is impossible to publish this revision without recognizing the visionary leadership of the previous BMBL editors, Drs. John Richardson and W. Emmett Barkley, and Drs. Jonathan Richmond and Robert W. McKinney, without whom the BMBL would not be the widely and well-regarded resource it is today. The Executive Steering Committee did a stellar job in shepherding this massive revision effort

and not without many bumps and bruises along the way. It is through their absolute commitment to quality, technical accuracy, and dedication to the professional practice of biosafety that the 5th edition is born. We are truly grateful to Ms. Kerstin Traum, Council Rock Consulting for her expertise, keen eye for detail and seemingly tireless efforts in performing the duties of technical writer-editor. We also gratefully acknowledge Ms. Cheryl Warfield of Proven Practices, LLC for her copy-editing and formatting skills that significantly enhanced this edition's readability and ease of use.

Finally, without the superb project management abilities and leadership of Dr. Joseph McDade and the technical/scientific editing expertise of Dr. Karl Johnson, especially in virology, the 5th edition of the BMBL would not be possible.

We hope you find this 5th edition of *Biosafety in Microbiological and Biomedical Laboratories* complete, timely and most of all, easy to use. Thank you for your patience and understanding during the long and comprehensive revision process. We believe you will find it was well worth the wait.

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BMBL is by its nature a continuously revised manual, and each revision refines and extends the contributions to previous editions. Since 1984, when the first edition of BMBL was published, many scientists and biosafety specialists have contributed to this important reference work. The 5th edition is no exception, as specialists in multiple disciplines generously provided their considerable expertise to this revision. The Editors and Steering Committee gratefully acknowledge the contributions of all of these many contributors over the life of the BMBL, especially contributors to the current edition, who are listed below.

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Section I—Introduction

Over the past two decades, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) has become the code of practice for biosafety—the discipline addressing the safe handling and containment of infectious microorganisms and hazardous biological materials. The principles of biosafety introduced in 1984 in the first edition of BMBL¹ and carried through in this fifth edition remain steadfast. These principles are containment and risk assessment. The fundamentals of containment include the microbiological practices, safety equipment, and facility safeguards that protect laboratory workers, the environment, and the public from exposure to infectious microorganisms that are handled and stored in the laboratory. Risk assessment is the process that enables the appropriate selection of microbiological practices, safety equipment, and facility safeguards that can prevent laboratory-associated infections (LAI). The purpose of periodic updates of BMBL is to refine guidance based on new knowledge and experiences and to address contemporary issues that present new risks that confront laboratory workers and the public health. In this way the code of practice will continue to serve the microbiological and biomedical community as a relevant and valuable authoritative reference.

We are living in an era of uncertainty and change. New infectious agents and diseases have emerged. Work with infectious agents in public and private research, public health, clinical and diagnostic laboratories, and in animal care facilities has expanded. Recent world events have demonstrated new threats of bioterrorism. For these reasons, organizations and laboratory directors are compelled to evaluate and ensure the effectiveness of their biosafety programs, the proficiency of their workers, as well as the capability of equipment, facilities, and management practices to provide containment and security of microbiological agents. Similarly, individual workers who handle pathogenic microorganisms must understand the containment conditions under which infectious agents can be safely manipulated and secured. Application of this knowledge and the use of appropriate techniques and equipment will enable the microbiological and biomedical community to prevent personal, laboratory and environmental exposure to potentially infectious agents or biohazards.

The Occurrence of Laboratory-Associated Infections

Published reports of LAIs first appeared around the start of the twentieth century. By 1978, four studies by Pike and Sulkin collectively identified 4,079 LAIs resulting in 168 deaths occurring between 1930 and 1978.²⁻⁵ These studies found that the ten most common causative agents of overt infections among workers were *Brucella* spp., *Coxiella burnetii*, hepatitis B virus (HBV), *Salmonella typhi*, *Francisella tularensis*, *Mycobacterium tuberculosis*, *Blastomyces dermatitidis*, Venezuelan equine encephalitis virus, *Chlamydia psittaci*, and *Coccidioides immitis*. The authors acknowledged that the 4,079 cases did not represent all LAIs that occurred during this period since many laboratories chose not to

report overt cases or conduct surveillance programs to identify sub-clinical or asymptomatic infections.

In addition, reports of LAIs seldom provided data sufficient to determine incidence rates, complicating quantitative assessments of risk. Similarly, there were no distinguishable accidents or exposure events identified in more than 80% of the LAIs reported before 1978. Studies did show that in many cases the infected person worked with a microbiological agent or was in the vicinity of another person handling an agent.²⁻⁶

During the 20 years following the Pike and Sulkin publications, a worldwide literature search by Harding and Byers revealed 1,267 overt infections with 22 deaths.⁷ Five deaths were of fetuses aborted as the consequence of a maternal LAI. *Mycobacterium tuberculosis*, *Coxiella burnetii*, hantavirus, arboviruses, HBV, *Brucella* spp., *Salmonella* spp., *Shigella* spp., hepatitis C virus, and *Cryptosporidium* spp. accounted for 1,074 of the 1,267 infections. The authors also identified an additional 663 cases that presented as sub-clinical infections. Like Pike and Sulkin, Harding and Byers reported that only a small number of the LAI involved a specific incident. The non-specific associations reported most often by these authors were working with a microbiological agent, being in or around the laboratory, or being around infected animals.

The findings of Harding and Byers indicated that clinical (diagnostic) and research laboratories accounted for 45% and 51%, respectively, of the total LAIs reported. This is a marked difference from the LAIs reported by Pike and Sulkin prior to 1979, which indicated that clinical and research laboratories accounted for 17% and 59%, respectively. The relative increase of LAIs in clinical laboratories may be due in part to improved employee health surveillance programs that are able to detect sub-clinical infections, or to the use of inadequate containment procedures during the early stages of culture identification.

Comparison of the more recent LAIs reported by Harding and Byers with those reported by Pike and Sulkin suggests that the number is decreasing. Harding and Byers note that improvements in containment equipment, engineering controls, and greater emphasis on safety training may be contributing factors to the apparent reduction in LAIs over two decades. However, due to the lack of information on the actual numbers of infections and the population at risk, it is difficult to determine the true incidence of LAIs with any degree of certainty.

Publication of the occurrence of LAIs provides an invaluable resource for the microbiological and biomedical community. For example, one report of occupational exposures associated with *Brucella melitensis*, an organism capable of transmission by the aerosol route, described how a staff member in a clinical microbiology laboratory accidentally sub-cultured *B. melitensis* on the open bench.⁸ This error and a breach in containment practices resulted in eight LAIs with *B. melitensis* among 26 laboratory members, an attack rate of 31%.

Reports of LAIs can serve as lessons in the importance of maintaining safe conditions in biological research.

Evolution of National Biosafety Guidelines

National biosafety guidelines evolved from the efforts of the microbiological and biomedical community to promote the use of safe microbiological practices, safety equipment and facility safeguards that will reduce LAIs and protect the public health and environment. The historical accounts of LAIs raised awareness about the hazards of infectious microorganisms and the health risks to laboratory workers who handle them. Many published accounts suggested practices and methods that might prevent LAIs.⁹ Arnold G. Wedum was the Director of Industrial Health and Safety at the United States Army Biological Research Laboratories, Fort Detrick from 1944 to 1969. His pioneering work in biosafety provided the foundation for evaluating the risks of handling infectious microorganisms and for recognizing biological hazards and developing practices, equipment, and facility safeguards for their control. Fort Detrick also advanced the field by aiding the development of biosafety programs at the United States Department of Agriculture (USDA), National Animal Research Center and the United States Department of Health and Human Services (DHHS), Centers for Disease Control and Prevention (CDC) and National Institutes of Health (NIH). These governmental organizations subsequently developed several national biosafety guidelines that preceded the first edition of BMBL.

In 1974, the CDC published *Classification of Etiologic Agents on the Basis of Hazard*.¹⁰ This report introduced the concept for establishing ascending levels of containment that correspond to risks associated with handling infectious microorganisms that present similar hazardous characteristics. Human pathogens were grouped into four classes according to mode of transmission and the severity of disease they caused. A fifth class included non-indigenous animal pathogens whose entry into the United States was restricted by USDA policy.

The NIH published *National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses* in 1974.¹¹ These guidelines established three levels of containment based on an assessment of the hypothetical risk of cancer in humans from exposure to animal oncogenic viruses or a suspected human oncogenic virus isolate from man.^{12,13} In 1976 NIH first published the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*.¹⁴ The *NIH Guidelines* described in detail the microbiological practices, equipment, and facility safeguards that correspond to four ascending levels of physical containment and established criteria for assigning experiments to a containment level based on an assessment of potential hazards of this emerging technology. The evolution of these guidelines set the foundation for developing a code of practice for biosafety in microbiological and biomedical laboratories. Led by the CDC and NIH, a broad collaborative initiative involving scientists, laboratory directors,

occupational physicians, epidemiologists, public health officials and health and safety professionals developed the first edition of BMBL in 1984. The BMBL provided the technical content not previously available in biosafety guidelines by adding summary statements conveying guidance pertinent to infectious microorganisms that had caused LAIs. The fifth edition of BMBL is also the product of a broad collaborative initiative committed to perpetuate the value of this national biosafety code of practice.

Risk Criteria for Establishing Ascending Levels of Containment

The primary risk criteria used to define the four ascending levels of containment, referred to as biosafety levels 1 through 4, are infectivity, severity of disease, transmissibility, and the nature of the work being conducted. Another important risk factor for agents that cause moderate to severe disease is the origin of the agent, whether indigenous or exotic. Each level of containment describes the microbiological practices, safety equipment and facility safeguards for the corresponding level of risk associated with handling a particular agent. The basic practices and equipment are appropriate for protocols common to most research and clinical laboratories. The facility safeguards help protect non-laboratory occupants of the building and the public health and environment.

Biosafety level 1 (BSL-1) is the basic level of protection and is appropriate for agents that are not known to cause disease in normal, healthy humans. Biosafety level 2 (BSL-2) is appropriate for handling moderate-risk agents that cause human disease of varying severity by ingestion or through percutaneous or mucous membrane exposure. Biosafety level 3 (BSL-3) is appropriate for agents with a known potential for aerosol transmission, for agents that may cause serious and potentially lethal infections and that are indigenous or exotic in origin. Exotic agents that pose a high individual risk of life-threatening disease by infectious aerosols and for which no treatment is available are restricted to high containment laboratories that meet biosafety level 4 (BSL-4) standards.

It is important to emphasize that the causative incident for most LAIs is unknown.^{7,8} Less obvious exposures such as the inhalation of infectious aerosols or direct contact of the broken skin or mucous membranes with droplets containing an infectious microorganism or surfaces contaminated by droplets may possibly explain the incident responsible for a number of LAIs. Most manipulations of liquid suspensions of microorganisms produce aerosols and droplets. Small-particle aerosols have respirable size particles that may contain one or several microorganisms. These small particles stay airborne and easily disperse throughout the laboratory. When inhaled, the human lung will retain those particles. Larger particle droplets rapidly fall out of the air, contaminating gloves, the immediate work area, and the mucous membranes of unprotected workers. A procedure's potential to release microorganisms into the air as aerosols and droplets is the most important operational risk factor that supports the need for containment equipment and facility safeguards.

Agent Summary Statements

The fifth edition, as in all previous editions, includes agent summary statements that describe the hazards, recommended precautions, and levels of containment appropriate for handling specific human and zoonotic pathogens in the laboratory and in facilities that house laboratory vertebrate animals. Agent summary statements are included for agents that meet one or more of the following three criteria:

1. the agent is a proven hazard to laboratory personnel working with infectious materials;
2. the agent has a high potential for causing LAIs even though no documented cases exist; and
3. the agent causes grave disease or presents a significant public health hazard.

Scientists, clinicians, and biosafety professionals prepared the statements by assessing the risks of handling the agents using standard protocols followed in many laboratories. **No one should conclude that the absence of an agent summary statement for a human pathogen means that the agent is safe to handle at BSL-1, or without a risk assessment to determine the appropriate level of containment.** Laboratory directors should also conduct independent risk assessments before beginning work with an agent or procedure new to the laboratory, even though an agent summary statement is available. There may be situations where a laboratory director should consider modifying the precautionary measures or recommended practices, equipment, and facility safeguards described in an agent summary statement. In addition, laboratory directors should seek guidance when conducting risk assessments. Knowledgeable colleagues; institutional biosafety committees; biosafety officers; and public health, biosafety, and scientific associations are excellent resources.

The agent summary statements in the fourth edition BMBL were reviewed in the course of preparing the fifth edition of BMBL. There are new and updated agent summary statements including those for agents now classified as Select Agents. For example, there is an updated section on arboviruses and related zoonotic viruses including new agent summary statements. There are also substantive revisions to the Influenza Agent Summary Statement that address non-contemporary human influenza strains and recommend safeguards for research involving reverse genetics of the 1918 influenza strain.

The fifth edition also includes a revised section on risk assessment that gives more emphasis on the importance of this process in selecting the appropriate practices and level of containment. That section intentionally follows this introduction because risk assessment represents the foundation—a code of practice for safe handling of infectious agents in microbiological and biomedical laboratories.

Biosecurity

Today, the nation is facing a new challenge in safeguarding the public health from potential domestic or international terrorism involving the use of dangerous biological agents or toxins. Existing standards and practices may require adaptation to ensure protection from such hostile actions. In addition, recent federal regulations mandate increased security within the microbiological and biomedical community in order to protect biological pathogens and toxins from theft, loss, or misuse. The fifth edition of BMBL includes an important new section on biosecurity—the discipline addressing the security of microbiological agents and toxins and the threats posed to human and animal health, the environment, and the economy by deliberate misuse or release. A careful review of the biosecurity concepts and guidelines introduced in this new section is essential for all laboratory workers.

Using BMBL

BMBL is both a code of practice and an authoritative reference. Knowledge sufficient to work safely with hazardous microorganisms requires a careful review of the entire BMBL. This will offer the reader an understanding of the biosafety principles that serve as the basis for the concepts and recommendations included in this reference. Reading only selected sections will not adequately prepare even an experienced laboratory worker to handle potentially infectious agents safely.

The recommended practices, safety equipment, and facility safeguards described in the first edition of BMBL and expanded in the fifth edition are advisory in most circumstances. The intent was and is to establish a voluntary code of practice, one that all members of a laboratory community will together embrace to safeguard themselves and their colleagues, and to protect the public health and environment.

Looking Ahead

Laboratory-associated infections from exposure to biological agents known to cause disease are infrequent. It is critical that the microbiological and biomedical community continue its resolve to remain vigilant and not to become complacent. The LAIs reported in the last 25 years demonstrate that accidents and unrecognized exposures continue to occur. The absence of clear evidence of the means of transmission in most documented LAI should motivate persons at risk to be alert to all potential routes of exposure. The accidental release of microbial aerosols is a probable cause of many LAI¹⁵, which demonstrates the importance of worker training and the ability to recognize potential hazards and correct unsafe habits. Attention to and proficient use of work practices, safety equipment and engineering controls are also essential.

The nation's response to recent world events brings with it a heightened concern for a potential increase in LAIs. In 2003, the United States federal government awarded significant funding for the construction of National Biocontainment Laboratories (NBL) and Regional Biocontainment Laboratories (RBL). The NBLs will house BSL-2, 3, and 4 laboratories; the RBLs will house BSL-2 and 3 laboratories. In addition, construction of new containment facilities by private and public institutions is underway nationwide. The expansion of biocontainment laboratories nationwide dramatically increases the need for training in microbiological practices and biosafety principles.

Understanding the principles of biosafety and adherence to the microbiological practices, containment and facility safeguards described in BMBL will contribute to a safer and healthier working environment for laboratory staff and adjacent personnel, and the community.

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Section II—Biological Risk Assessment

Risk assessment is an important responsibility for directors and principal investigators of microbiological and biomedical laboratories. Institutional biosafety committees (IBC), animal care and use committees, biological safety professionals, and laboratory animal veterinarians share in this responsibility. Risk assessment is a process used to identify the hazardous characteristics of a known infectious or potentially infectious agent or material, the activities that can result in a person's exposure to an agent, the likelihood that such exposure will cause a LAI, and the probable consequences of such an infection. The information identified by risk assessment will provide a guide for the selection of appropriate biosafety levels and microbiological practices, safety equipment, and facility safeguards that can prevent LAIs.

Laboratory directors and principal investigators should use risk assessment to alert their staffs to the hazards of working with infectious agents and to the need for developing proficiency in the use of selected safe practices and containment equipment. Successful control of hazards in the laboratory also protects persons not directly associated with the laboratory, such as other occupants of the same building, and the public.

Risk assessment requires careful judgment. Adverse consequences are more likely to occur if the risks are underestimated. By contrast, imposition of safeguards more rigorous than actually needed may result in additional expense and burden for the laboratory, with little safety enhancement. Unnecessary burden may result in circumvention of required safeguards. However, where there is insufficient information to make a clear determination of risk, it is prudent to consider the need for additional safeguards until more data are available.

The primary factors to consider in risk assessment and selection of precautions fall into two broad categories: agent hazards and laboratory procedure hazards. In addition, the capability of the laboratory staff to control hazards must be considered. This capability will depend on the training, technical proficiency, and good habits of all members of the laboratory, and the operational integrity of containment equipment and facility safeguards.

The agent summary statements contained in BMBL identify the primary agent and procedure hazards for specific pathogens and recommend precautions for their control. The guest editors and contributors of this and previous editions of BMBL based their recommendations on an assessment of the risks associated with the handling of pathogens using generally routine generic laboratory procedures. A review of the summary statement for a specific pathogen is a helpful starting point for assessment of the risks of working with that agent and those for a similar agent.

Hazardous Characteristics of an Agent

The principal hazardous characteristics of an agent are: its capability to infect and cause disease in a susceptible human or animal host, its virulence as measured by the severity of disease, and the availability of preventive measures and effective treatments for the disease. The World Health Organization (WHO) has recommended an agent risk group classification for laboratory use that describes four general risk groups based on these principal characteristics and the route of transmission of the natural disease.¹ The four groups address the risk to both the laboratory worker and the community. The *NIH Guidelines* established a comparable classification and assigned human etiological agents into four risk groups on the basis of hazard.² The descriptions of the WHO and NIH risk group classifications are presented in Table 1. They correlate with but do not equate to biosafety levels. A risk assessment will determine the degree of correlation between an agent's risk group classification and biosafety level. See Section 3 for a further discussion of the differences and relatedness of risk groups and biosafety levels.

Table 1: Classification of Infectious Microorganisms by Risk Group

Risk Group Classification	NIH Guidelines for Research involving Recombinant DNA Molecules 2002²	World Health Organization Laboratory Biosafety Manual 3rd Edition 2004¹
Risk Group 1	Agents not associated with disease in healthy adult humans.	(No or low individual and community risk) A microorganism unlikely to cause human or animal disease.
Risk Group 2	Agents associated with human disease that is rarely serious and for which preventive or therapeutic interventions are <i>often</i> available.	(Moderate individual risk; low community risk) A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.
Risk Group 3	Agents associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk).	(High individual risk; low community risk) A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.
Risk Group 4	Agents likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk).	(High individual and community risk) A pathogen that usually causes serious human or animal disease and can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available. ³

Other hazardous characteristics of an agent include probable routes of transmission of laboratory infection, infective dose, stability in the environment, host range, and its endemic nature. In addition, reports of LAIs are a clear indicator of hazard and often are sources of information helpful for identifying agent and procedural hazards, and the precautions for their control. The absence of a report does not indicate minimal risk. Reports seldom provide incidence data, making comparative judgments on risks among agents difficult. The number of infections reported for a single agent may be an indication of the frequency of use as well as risk. Nevertheless, reporting of LAIs by laboratory directors in the scientific and medical literature is encouraged. Reviews of such reports and analyses of LAIs identified through extensive surveys are a valuable resource for risk assessment and reinforcement of the biosafety principles. The summary statements in BMBL include specific references to reports on LAIs.

The predominant probable routes of transmission in the laboratory are: 1) direct skin, eye or mucosal membrane exposure to an agent; 2) parenteral inoculation by a syringe needle or other contaminated sharp, or by bites from infected animals and arthropod vectors; 3) ingestion of liquid suspension of an infectious agent, or by contaminated hand to mouth exposure; and 4) inhalation of infectious aerosols. An awareness of the routes of transmission for the natural human disease is helpful in identifying probable routes of transmission in the laboratory and the potential for any risk to the public health. For example, transmission of infectious agents can occur by direct contact with discharges from respiratory mucous membranes of infected persons, which would be a clear indication that a laboratory worker is at risk of infection from mucosal membrane exposure to droplets generated while handling that agent. The American Public Health Association publication *Control of Communicable Diseases Manual* is an excellent reference for identifying both natural and often noted laboratory modes of transmission.³ However, it is important to remember that the nature and severity of disease caused by a laboratory infection and the probable laboratory route of transmission of the infectious agent may differ from the route of transmission and severity associated with the naturally-acquired disease.⁴

An agent capable of transmitting disease through respiratory exposure to infectious aerosols is a serious laboratory hazard, both for the person handling the agent and for other laboratory occupants. This hazard requires special caution because infectious aerosols may not be a recognized route of transmission for the natural disease. Infective dose and agent stability are particularly important in establishing the risk of airborne transmission of disease. For example, the reports of multiple infections in laboratories associated with the use of *Coxiella burnetii* are explained by its low inhalation infective dose, which is estimated to be ten inhaled infectious particles, and its resistance to environmental stresses that enables the agent to survive outside of a living host or culture media long enough to become an aerosol hazard.⁵

When work involves the use of laboratory animals, the hazardous characteristics of zoonotic agents require careful consideration in risk assessment. Evidence that experimental animals can shed zoonotic agents and other infectious agents under study in saliva, urine, or feces is an important indicator of hazard. The death of a primate center laboratory worker from Cercopithecine herpes virus 1 (CHV-1, also known as Monkey B virus) infection following an ocular splash exposure to biologic material from a rhesus macaque emphasizes the seriousness of this hazard.⁶ Lack of awareness for this potential hazard can make laboratory staff vulnerable to an unexpected outbreak involving multiple infections.⁷ Experiments that demonstrate transmission of disease from an infected animal to a normal animal housed in the same cage are reliable indicators of hazard. Experiments that do not demonstrate transmission, however, do not rule out hazard. For example, experimental animals infected with *Francisella tularensis*, *Coxiella burnetii*, *Coccidioides immitis*, or *Chlamydia psittaci*—agents that have caused many LAIs—rarely infect cagemates.⁸

The origin of the agent is also important in risk assessment. Non-indigenous agents are of special concern because of their potential to introduce risk of transmission, or spread of human and animal or infectious diseases from foreign countries into the United States. Importation of etiological agents of human disease requires a permit from the CDC. Importation of many etiological agents of livestock, poultry and other animal diseases requires a permit from the USDA's Animal and Plant Health Inspection Service (APHIS). For additional details, see Appendix C.

Genetically modified agent hazards. The identification and assessment of hazardous characteristics of genetically modified agents involve consideration of the same factors used in risk assessment of the wild-type organism. It is particularly important to address the possibility that the genetic modification could increase an agent's pathogenicity or affect its susceptibility to antibiotics or other effective treatments. The risk assessment can be difficult or incomplete, because important information may not be available for a newly engineered agent. Several investigators have reported that they observed unanticipated enhanced virulence in recent studies with engineered agents.⁹⁻¹² These observations give reason to remain alert to the possibility that experimental alteration of virulence genes may lead to increased risk. It also suggests that risk assessment is a continuing process that requires updating as research progresses.

The *NIH Guidelines* are the key reference in assessing risk and establishing an appropriate biosafety level for work involving recombinant DNA molecules.² The purpose of the *NIH Guidelines* is to promote the safe conduct of research involving recombinant DNA. The guidelines specify appropriate practices and procedures for research involving constructing and handling both recombinant DNA molecules and organisms and viruses that contain recombinant DNA. They define recombinant DNA as a molecule constructed outside of a living cell with the capability to replicate in a living cell. The *NIH Guidelines* explicitly address experiments that involve introduction of recombinant DNA into Risk Groups 2, 3,

and 4 agents, and experiments in which the DNA from Risk Groups 2, 3, and 4 agents is cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector systems. Compliance with the *NIH Guidelines* is mandatory for investigators conducting recombinant DNA research funded by the NIH or performed at, or sponsored by, any public or private entity that receives any NIH funding for recombinant DNA research. Many other institutions have adopted these guidelines as the best current practice.

The *NIH Guidelines* were first published in 1976 and are revised on an ongoing basis in response to scientific and policy developments. The guidelines outline the roles and responsibilities of various entities affiliated with recombinant DNA research, including institutions, investigators, and the NIH. Recombinant DNA research subject to the *NIH Guidelines* may require: 1) approval by the NIH Director, review by the NIH Recombinant DNA Advisory Committee (RAC), and approval by the IBC; or 2) review by the NIH Office of Biotechnology Activities (OBA) and approval by the IBC; or 3) review by the RAC and approvals by the IBC and Institutional Review Board; or 4) approval by the IBC prior to initiation of the research; or 5) notification of the IBC simultaneous with initiation of the work. It is important to note that review by an IBC is required for all non-exempt experiments as defined by the *NIH Guidelines*.

The *NIH Guidelines* were the first documents to formulate the concept of an IBC as the responsible entity for biosafety issues stemming from recombinant DNA research. The *NIH Guidelines* outline the membership, procedures, and functions of an IBC. The institution is ultimately responsible for the effectiveness of the IBC, and may define additional roles and responsibilities for the IBC apart from those specified in the *NIH Guidelines*. See Appendix J for more information about the *NIH Guidelines* and OBA.

Cell cultures. Workers who handle or manipulate human or animal cells and tissues are at risk for possible exposure to potentially infectious latent and adventitious agents that may be present in those cells and tissues. This risk is well understood and illustrated by the reactivation of herpes viruses from latency,^{13,14} the inadvertent transmission of disease to organ recipients,^{15,16} and the persistence of human immunodeficiency virus (HIV), HBV, and hepatitis C virus (HCV) within infected individuals in the U.S. population.¹⁷ There also is evidence of accidental transplantation of human tumor cells to healthy recipients which indicates that these cells are potentially hazardous to laboratory workers who handle them.¹⁸ In addition, human and animal cell lines that are not well characterized or are obtained from secondary sources may introduce an infectious hazard to the laboratory. For example, the handling of nude mice inoculated with a tumor cell line unknowingly infected with lymphocytic choriomeningitis virus resulted in multiple LAIs.¹⁹ The potential for human cell lines to harbor a bloodborne pathogen led the Occupational Health and Safety Administration (OSHA) to interpret that the occupational exposure to bloodborne pathogens final rule would include primary human cell lines and explants.¹⁷

Hazardous Characteristics of Laboratory Procedures

Investigations of LAIs have identified five principal routes of laboratory transmission. These are parenteral inoculations with syringe needles or other contaminated sharps, spills and splashes onto skin and mucous membranes, ingestion through mouth pipetting, animal bites and scratches, and inhalation exposures to infectious aerosols. The first four routes of laboratory transmission are easy to detect, but account for less than 20 percent of all reported LAIs.²⁰ Most reports of such infections do not include information sufficient to identify the route of transmission of infection. Work has shown that the probable sources of infection—animal or ectoparasite, clinical specimen, agent, and aerosol—are apparent in approximately 50 percent of cases.²¹

Aerosols are a serious hazard because they are ubiquitous in laboratory procedures, are usually undetected, and are extremely pervasive, placing the laboratory worker carrying out the procedure and other persons in the laboratory at risk of infection. There is general agreement among biosafety professionals, laboratory directors and principal investigators who have investigated LAIs that an aerosol generated by procedures and operations is the probable source of many LAIs, particularly in cases involving workers whose only known risk factor was that they worked with an agent or in an area where that work was done.

Procedures that impart energy to a microbial suspension will produce aerosols. Procedures and equipment used routinely for handling infectious agents in laboratories, such as pipetting, blenders, non-self contained centrifuges, sonicators and vortex mixers are proven sources of aerosols. These procedures and equipment generate respirable-size particles that remain airborne for protracted periods. When inhaled, these particles are retained in the lungs creating an exposure hazard for the person performing the operation, coworkers in the laboratory, and a potential hazard for persons occupying adjacent spaces open to air flow from the laboratory. A number of investigators have determined the aerosol output of common laboratory procedures. In addition, investigators have proposed a model for estimating inhalation dosage from a laboratory aerosol source. Parameters that characterize aerosol hazards include an agent's inhalation infective dose, its viability in an aerosol, aerosol concentration, and particle size.^{22,23,24}

Procedures and equipment that generate respirable size particles also generate larger size droplets that can contain multiple copies of an infectious agent. The larger size droplets settle out of the air rapidly, contaminating the gloved hands and work surface and possibly the mucous membranes of the persons performing the procedure. An evaluation of the release of both respirable particles and droplets from laboratory operations determined that the respirable component is relatively small and does not vary widely; in contrast hand and surface contamination is substantial and varies widely.²⁵ The potential risk from exposure to droplet contamination requires as much attention in a risk assessment as the respirable component of aerosols.

Technique can significantly impact aerosol output and dose. The worker who is careful and proficient will minimize the generation of aerosols. A careless and hurried worker will substantially increase the aerosol hazard. For example, the hurried worker may operate a sonic homogenizer with maximum aeration whereas the careful worker will consistently operate the device to assure minimal aeration. Experiments show that the aerosol burden with maximal aeration is approximately 200 times greater than aerosol burden with minimal aeration.²² Similar results were shown for pipetting with bubbles and with minimal bubbles. Containment and good laboratory practices also reduce this risk.

Potential Hazards Associated with Work Practices, Safety Equipment and Facility Safeguards

Workers are the first line of defense for protecting themselves, others in the laboratory, and the public from exposure to hazardous agents. Protection depends on the conscientious and proficient use of good microbiological practices and the correct use of safety equipment. A risk assessment should identify any potential deficiencies in the practices of the laboratory workers. Carelessness is the most serious concern, because it can compromise any safeguards of the laboratory and increase the risk for coworkers. Training, experience, knowledge of the agent and procedure hazards, good habits, caution, attentiveness, and concern for the health of coworkers are prerequisites for a laboratory staff in order to reduce the inherent risks that attend work with hazardous agents. Not all workers who join a laboratory staff will have these prerequisite traits even though they may possess excellent scientific credentials. Laboratory directors or principal investigators should train and retrain new staff to the point where aseptic techniques and safety precautions become second nature.²⁶

There may be hazards that require specialized personal protective equipment in addition to safety glasses, laboratory gowns, and gloves. For example, a procedure that presents a splash hazard may require the use of a mask and a face shield to provide adequate protection. Inadequate training in the proper use of personal protective equipment may reduce its effectiveness, provide a false sense of security, and could increase the risk to the laboratory worker. For example, a respirator may impart a risk to the wearer independent of the agents being manipulated.

Safety equipment such as biological safety cabinets (BSC), centrifuge safety cups, and sealed rotors are used to provide a high degree of protection for the laboratory worker from exposure to microbial aerosols and droplets. Safety equipment that is not working properly is hazardous, especially when the user is unaware of the malfunction. Poor location, room air currents, decreased airflow, leaking filters, raised sashes, crowded work surfaces, and poor user technique compromise the containment capability of a BSC. The safety characteristics of modern centrifuges are only effective if the equipment is operated properly. Training in the correct use of equipment, proper procedure, routine inspections

and potential malfunctions, and periodic re-certification of equipment, as needed, is essential.

Facility safeguards help prevent the accidental release of an agent from the laboratory. Their use is particularly important at BSL-3 and BSL-4 because the agents assigned to those levels can transmit disease by the inhalation route or can cause life-threatening disease. For example, one facility safeguard is directional airflow. This safeguard helps to prevent aerosol transmission from a laboratory into other areas of the building. Directional airflow is dependent on the operational integrity of the laboratory's heating, ventilation, and air conditioning (HVAC) system. HVAC systems require careful monitoring and periodic maintenance to sustain operational integrity. Loss of directional airflow compromises safe laboratory operation. BSL-4 containment facilities provide more complex safeguards that require significant expertise to design and operate.

Consideration of facility safeguards is an integral part of the risk assessments. A biological safety professional, building and facilities staff, and the IBC should help assess the facility's capability to provide appropriate protection for the planned work, and recommend changes as necessary. Risk assessment may support the need to include additional facility safeguards in the construction of new or renovation of old BSL-3 facilities.

An Approach to Assess Risks and Select Appropriate Safeguards

Biological risk assessment is a subjective process requiring consideration of many hazardous characteristics of agents and procedures, with judgments based often on incomplete information. There is no standard approach for conducting a biological risk assessment, but some structure can be helpful in guiding the process. This section describes a five-step approach that gives structure to the risk assessment process.

First, identify agent hazards and perform an initial assessment of risk.

Consider the principal hazardous characteristics of the agent, which include its capability to infect and cause disease in a susceptible human host, severity of disease, and the availability of preventive measures and effective treatments.

Several excellent resources provide information and guidance for making an initial risk assessment. The BMBL provides agent summary statements for some agents that are associated with LAIs or are of increased public concern. Agent summary statements also identify known and suspected routes of transmission of laboratory infection and, when available, information on infective dose, host range, agent stability in the environment, protective immunizations, and attenuated strains of the agent.

A thorough examination of the agent hazards is necessary when the intended use of an agent does not correspond with the general conditions described in the summary statement or when an agent summary statement is

not available. Although a summary statement for one agent may provide helpful information for assessing the risk of a similar agent, it should not serve as the primary resource for making the risk determination for that agent. Refer to other resources for guidance in identifying the agent hazards.

The *Control of Communicable Diseases Manual* provides information on communicable diseases including concise summaries on severity, mode of transmission, and the susceptibility and resistance of humans to disease.³ In addition, it is always helpful to seek guidance from colleagues with experience in handling the agent and from biological safety professionals.

Often there is not sufficient information to make an appropriate assessment of risk. For example, the hazard of an unknown agent that may be present in a diagnostic specimen will be unknown until after completing agent identification and typing procedures. It would be prudent in this case to assume the specimen contains an agent presenting the hazardous classification that correlates with BSL-2, unless additional information suggests the presence of an agent of higher risk. Identification of agent hazards associated with newly emergent pathogens also requires judgments based on incomplete information. Consult interim biosafety guidelines prepared by the CDC and the WHO for risk assessment guidance. When assessing the hazards of a newly attenuated pathogen, experimental data should support a judgment that the attenuated pathogen is less hazardous than the wild-type parent pathogen before making any reduction in the containment recommended for that pathogen.

Make a preliminary determination of the biosafety level that best correlates with the initial risk assessment based on the identification and evaluation of the agent hazards. Remember that aerosol and droplet routes of agent transmission also are important considerations in specification of safety equipment and facility design that result in a given BSL level.

Second, identify laboratory procedure hazards. The principal laboratory procedure hazards are agent concentration, suspension volume, equipment and procedures that generate small particle aerosols and larger airborne particles (droplets), and use of sharps. Procedures involving animals can present a number of hazards such as bites and scratches, exposure to zoonotic agents, and the handling of experimentally generated infectious aerosols.

The complexity of a laboratory procedure can also present a hazard. The agent summary statement provides information on the primary laboratory hazards associated with typically routine procedures used in handling an agent. In proposed laboratory procedures where the procedure hazards differ from the general conditions of the agent summary statement or where an agent summary statement is not available, the risk assessment should identify specific hazards associated with the procedures.

Third, make a determination of the appropriate biosafety level and select additional precautions indicated by the risk assessment. The selection of the appropriate biosafety level and the selection of any additional laboratory precautions require a comprehensive understanding of the practices, safety equipment, and facility safeguards described in Sections III, IV, and V of this publication.

There will be situations where the intended use of an agent requires greater precautions than those described in the agent's Summary Statement. These situations will require the careful selection of additional precautions. An obvious example would be a procedure for exposing animals to experimentally generated infectious aerosols.

It is unlikely that a risk assessment would indicate a need to alter the recommended facility safeguards specified for the selected biosafety level. If this does occur, however, it is important that a biological safety professional validate this judgment independently before augmenting any facility secondary barrier.

It is also important to recognize that individuals in the laboratory may differ in their susceptibility to disease. Pre-existing diseases, medications, compromised immunity, and pregnancy or breast-feeding that may increase exposure to infants to certain agents, are some of the conditions that may increase the risk of an individual for acquiring a LAI. Consultation with an occupational physician knowledgeable in infectious diseases is advisable in these circumstances.

Fourth, evaluate the proficiencies of staff regarding safe practices and the integrity of safety equipment. The protection of laboratory workers, other persons associated with the laboratory, and the public will depend ultimately on the laboratory workers themselves. In conducting a risk assessment, the laboratory director or principal investigator should ensure that laboratory workers have acquired the technical proficiency in the use of microbiological practices and safety equipment required for the safe handling of the agent, and have developed good habits that sustain excellence in the performance of those practices. An evaluation of a person's training, experience in handling infectious agents, proficiency in the use of sterile techniques and BSCs, ability to respond to emergencies, and willingness to accept responsibility for protecting one's self and others is important insurance that a laboratory worker is capable of working safely.

The laboratory director or principal investigator should also ensure that the necessary safety equipment is available and operating properly. For example, a BSC that is not certified represents a potentially serious hazard to the laboratory worker using it and to others in the laboratory. The director should have all equipment deficiencies corrected before starting work with an agent.

Fifth, review the risk assessment with a biosafety professional, subject matter expert, and the IBC. A review of the risk assessment and selected safeguards by knowledgeable individuals is always beneficial and sometimes required by regulatory or funding agencies, as is the case with the *NIH Guidelines*.² Review of potentially high risk protocols by the local IBC should become standard practice. Adopting this step voluntarily will promote the use of safe practices in work with hazardous agents in microbiological and biomedical laboratories.

Conclusion

Risk assessment is the basis for the safeguards developed by the CDC, the NIH, and the microbiological and biomedical community to protect the health of laboratory workers and the public from the risks associated with the use of hazardous biological agents in laboratories. Experience shows that these established safe practices, equipment, and facility safeguards work.

New knowledge and experience may justify altering these safeguards. Risk assessment, however, must be the basis for recommended change. Assessments conducted by laboratory directors and principal investigators for the use of emergent agents and the conduct of novel experiments will contribute to our understanding of the risks these endeavors may present and the means for their control. Those risk assessments will likely mirror progress in science and technology and serve as the basis for future revisions of BMBL.

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Section III—Principles of Biosafety

A fundamental objective of any biosafety program is the containment of potentially harmful biological agents. The term “containment” is used in describing safe methods, facilities and equipment for managing infectious materials in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents. The use of vaccines may provide an increased level of personal protection. The risk assessment of the work to be done with a specific agent will determine the appropriate combination of these elements.

Laboratory Practices and Technique

The most important element of containment is strict adherence to standard microbiological practices and techniques. Persons working with infectious agents or potentially infected materials must be aware of potential hazards, and must be trained and proficient in the practices and techniques required for handling such material safely. The director or person in charge of the laboratory is responsible for providing or arranging the appropriate training of personnel.

Each laboratory should develop or adopt a biosafety or operations manual that identifies the hazards that will or may be encountered, and that specifies practices and procedures designed to minimize or eliminate exposures to these hazards. Personnel should be advised of special hazards and should be required to read and follow the required practices and procedures. A scientist, trained and knowledgeable in appropriate laboratory techniques, safety procedures, and hazards associated with handling infectious agents must be responsible for the conduct of work with any infectious agents or materials. This individual should consult with biosafety or other health and safety professionals with regard to risk assessment.

When standard laboratory practices are not sufficient to control the hazards associated with a particular agent or laboratory procedure, additional measures may be needed. The laboratory director is responsible for selecting additional safety practices, which must be in keeping with the hazards associated with the agent or procedure.

Appropriate facility design and engineering features, safety equipment, and management practices must supplement laboratory personnel, safety practices, and techniques.

Safety Equipment (Primary Barriers and Personal Protective Equipment)

Safety equipment includes BSCs, enclosed containers, and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The BSC is the principal device used to provide containment of

infectious droplets or aerosols generated by many microbiological procedures. Three types of BSCs (Class I, II, III) used in microbiological laboratories are described and illustrated in Appendix A. Open-fronted Class I and Class II BSCs are primary barriers that offer significant levels of protection to laboratory personnel and to the environment when used with good microbiological techniques. The Class II biological safety cabinet also provides protection from external contamination of the materials (e.g., cell cultures, microbiological stocks) being manipulated inside the cabinet. The gas-tight Class III biological safety cabinet provides the highest attainable level of protection to personnel and the environment.

An example of another primary barrier is the safety centrifuge cup, an enclosed container designed to prevent aerosols from being released during centrifugation. To minimize aerosol hazards, containment controls such as BSCs or centrifuge cups must be used when handling infectious agents.

Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Personal protective equipment is often used in combination with BSCs and other devices that contain the agents, animals, or materials being handled. In some situations in which it is impractical to work in BSCs, personal protective equipment may form the primary barrier between personnel and the infectious materials. Examples include certain animal studies, animal necropsy, agent production activities, and activities relating to maintenance, service, or support of the laboratory facility.

Facility Design and Construction (Secondary Barriers)

The design and construction of the facility contributes to the laboratory workers' protection, provides a barrier to protect persons outside the laboratory, and protects persons or animals in the community from infectious agents that may be accidentally released from the laboratory. Laboratory directors are responsible for providing facilities commensurate with the laboratory's function and the recommended biosafety level for the agents being manipulated.

The recommended secondary barrier(s) will depend on the risk of transmission of specific agents. For example, the exposure risks for most laboratory work in BSL-1 and BSL-2 facilities will be direct contact with the agents, or inadvertent contact exposures through contaminated work environments. Secondary barriers in these laboratories may include separation of the laboratory work area from public access, availability of a decontamination facility (e.g., autoclave), and hand washing facilities.

When the risk of infection by exposure to an infectious aerosol is present, higher levels of primary containment and multiple secondary barriers may become necessary to prevent infectious agents from escaping into the environment. Such design features include specialized ventilation systems to

ensure directional airflow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks at laboratory entrances, or separate buildings or modules to isolate the laboratory. Design engineers for laboratories may refer to specific ventilation recommendations as found in the ASHRAE Laboratory Design Guide published by the American Society of Heating, Refrigerating, and Air-Conditioning Engineers (ASHRAE).¹

Biosafety Levels

Four BSLs are described in Section 4, which consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the operations performed, the documented or suspected routes of transmission of the infectious agents, and the laboratory function or activity. The BSLs described in this manual should be differentiated from Risk Groups, as described in the *NIH Guidelines* and the World Health Organization Laboratory Biosafety Manual. Risk groups are the result of a classification of microbiological agents based on their association with, and resulting severity of, disease in humans. The risk group of an agent should be one factor considered in association with mode of transmission, procedural protocols, experience of staff, and other factors in determining the BSL in which the work will be conducted.

The recommended biosafety level(s) for the organisms in Section VIII (Agent Summary Statements) represent those conditions under which the agent ordinarily can be safely handled. Of course, not all of the organisms capable of causing disease are included in Section VIII and an institution must be prepared to perform risk assessments for these agents using the best available information. Detailed information regarding the conduct of biological risk assessments can be found in Section II. The laboratory director is specifically and primarily responsible for assessing the risks and applying the appropriate biosafety levels. The institution's Biological Safety Officer (BSO) and IBC can be of great assistance in performing and reviewing the required risk assessment. At one point, under the *NIH Guidelines*, BSOs were required only when large-scale research or production of organisms containing recombinant DNA molecules was performed or when work with recombinant DNA molecules was conducted at BSL-3 or above. IBCs were required only when an institution was performing non-exempt recombinant DNA experiments. Today, however, it is strongly suggested that an institution conducting research or otherwise working with pathogenic agents have a BSO and properly constituted and functioning IBC. The responsibilities of each now extend beyond those described in the *NIH Guidelines* and depend on the size and complexity of the program.

Generally, work with known agents should be conducted at the biosafety level recommended in Section VIII. When information is available to suggest that virulence, pathogenicity, antibiotic resistance patterns, vaccine and treatment availability, or other factors are significantly altered, more (or less) stringent

practices may be specified. Often an increased volume or a high concentration of agent may require additional containment practices.

Biosafety Level 1 practices, safety equipment, and facility design and construction are appropriate for undergraduate and secondary educational training and teaching laboratories, and for other laboratories in which work is done with defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans. *Bacillus subtilis*, *Nigeria gruberi*, infectious canine hepatitis virus, and exempt organisms under the *NIH Guidelines* are representative of microorganisms meeting these criteria. Many agents not ordinarily associated with disease processes in humans are, however, opportunistic pathogens and may cause infection in the young, the aged, and immunodeficient or immunosuppressed individuals. Vaccine strains that have undergone multiple *in vivo* passages should not be considered avirulent simply because they are vaccine strains.

BSL-1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for hand washing.

Biosafety Level 2 practices, equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, and other laboratories in which work is done with the broad spectrum of indigenous moderate-risk agents that are present in the community and associated with human disease of varying severity. With good microbiological techniques, these agents can be used safely in activities conducted on the open bench, provided the potential for producing splashes or aerosols is low. Hepatitis B virus, HIV, the *Salmonella*, and *Toxoplasma* are representative of microorganisms assigned to this containment level. BSL-2 is appropriate when work is done with any human-derived blood, body fluids, tissues, or primary human cell lines where the presence of an infectious agent may be unknown. (Laboratory personnel working with human-derived materials should refer to the OSHA Bloodborne Pathogen Standard² for specific required precautions).

Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials. Extreme caution should be taken with contaminated needles or sharp instruments. Even though organisms routinely manipulated at BSL-2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment, or in devices such as a BSC or safety centrifuge cups. Personal protective equipment should be used as appropriate, such as splash shields, face protection, gowns, and gloves.

Secondary barriers, such as hand washing sinks and waste decontamination facilities, must be available to reduce potential environmental contamination.

Biosafety Level 3 practices, safety equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. *Mycobacterium tuberculosis*, St. Louis encephalitis virus, and *Coxiella burnetii* are representative of the microorganisms assigned to this level. Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols. At BSL-3, more emphasis is placed on primary and secondary barriers to protect personnel in contiguous areas, the community, and the environment from exposure to potentially infectious aerosols. For example, all laboratory manipulations should be performed in a BSC or other enclosed equipment, such as a gas-tight aerosol generation chamber. Secondary barriers for this level include controlled access to the laboratory and ventilation requirements that minimize the release of infectious aerosols from the laboratory.

Biosafety Level 4 practices, safety equipment, and facility design and construction are applicable for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy. Agents with a close or identical antigenic relationship to BSL-4 agents also should be handled at this level. When sufficient data are obtained, work with these agents may continue at this level or at a lower level. Viruses such as Marburg or Congo-Crimean hemorrhagic fever are manipulated at BSL-4.

The primary hazards to personnel working with BSL-4 agents are respiratory exposure to infectious aerosols, mucous membrane or broken skin exposure to infectious droplets, and autoinoculation. All manipulations of potentially infectious diagnostic materials, isolates, and naturally or experimentally infected animals, pose a high risk of exposure and infection to laboratory personnel, the community, and the environment.

The laboratory worker's complete isolation from aerosolized infectious materials is accomplished primarily by working in a Class III BSC or in a full-body, air-supplied positive-pressure personnel suit. The BSL-4 facility itself is generally a separate building or completely isolated zone with complex, specialized ventilation requirements and waste management systems to prevent release of viable agents to the environment.

The laboratory director is specifically and primarily responsible for the safe operation of the laboratory. His/her knowledge and judgment are critical in assessing risks and appropriately applying these recommendations. The recommended biosafety level represents those conditions under which the agent can ordinarily be safely handled. Special characteristics of the agents used, the training and experience of personnel, procedures being conducted and the nature or function of the laboratory may further influence the director in applying these recommendations.

Animal Facilities

Four standard biosafety levels are also described for activities involving infectious disease work with commonly used experimental animals. These four combinations of practices, safety equipment, and facilities are designated Animal Biosafety Levels 1, 2, 3, and 4, and provide increasing levels of protection to personnel and the environment.

One additional biosafety level, designated BSL-3-Agriculture (or BSL 3-Ag) addresses activities involving large or loose-housed animals and/or studies involving agents designated as High Consequence Pathogens by the USDA. BSL 3-Ag laboratories are designed so that the laboratory facility itself acts as a primary barrier to prevent release of infectious agents into the environment. More information on the design and operation of BSL 3-Ag facilities and USDA High Consequence Pathogens is provided in Appendix D.

Clinical Laboratories

Clinical laboratories, especially those in health care facilities, receive clinical specimens with requests for a variety of diagnostic and clinical support services. Typically, the infectious nature of clinical material is unknown, and specimens are often submitted with a broad request for microbiological examination for multiple agents (e.g., sputa submitted for “routine,” acid-fast, and fungal cultures). It is the responsibility of the laboratory director to establish standard procedures in the laboratory that realistically address the issue of the infective hazard of clinical specimens.

Except in extraordinary circumstances (e.g., suspected hemorrhagic fever), the initial processing of clinical specimens and serological identification of isolates can be done safely at BSL-2, the recommended level for work with bloodborne pathogens such as HBV and HIV. The containment elements described in BSL-2 are consistent with the OSHA standard, “Occupational Exposure to Bloodborne Pathogens.”^{2,3} This requires the use of specific precautions with all clinical specimens of blood or other potentially infectious material (Universal or Standard* Precautions).^{4,5} Additionally, other recommendations specific for clinical laboratories may be obtained from the Clinical Laboratory Standards Institute (formerly known as the National Committee for Clinical Laboratory Standards).⁶

BSL-2 recommendations and OSHA requirements focus on the prevention of percutaneous and mucous membrane exposures to clinical material. Primary barriers such as BSCs (Class I or II) should be used when performing procedures that might cause splashing, spraying, or splattering of droplets. Biological safety cabinets also should be used for the initial processing of clinical specimens when the nature of the test requested or other information suggests the likely presence of an agent readily transmissible by infectious aerosols (e.g., *M. tuberculosis*), or when the use of a BSC (Class II) is indicated to protect the integrity of the specimen.

The segregation of clinical laboratory functions and limited or restricted access to such areas is the responsibility of the laboratory director. It is also the director's responsibility to establish standard, written procedures that address the potential hazards and the required precautions to be implemented.

Importation and Interstate Shipment of Certain Biomedical Materials

The importation of etiologic agents and vectors of human diseases is subject to the requirements of the Public Health Service Foreign Quarantine regulations. Companion regulations of the Public Health Service and the Department of Transportation specify packaging, labeling, and shipping requirements for etiologic agents and diagnostic specimens shipped in interstate commerce. (See Appendix C.)

The USDA regulates the importation and interstate shipment of animal pathogens and prohibits the importation, possession, or use of certain exotic animal disease agents that pose a serious disease threat to domestic livestock and poultry. (See Appendix F.)

Select Agents

In recent years, with the passing of federal legislation regulating the possession, use, and transfer of agents with high adverse public health and/or agricultural consequences (DHHS and USDA Select Agents), much greater emphasis has been placed in the emerging field of biosecurity. Biosecurity and select agent issues are covered in detail in Section 6 and Appendix F of this document. In contrast with biosafety, a field dedicated to the protection of workers and the environment from exposures to infectious materials, the field of biosecurity prevents loss of valuable research materials and limits access to infectious materials by individuals who would use them for harmful purposes. Nevertheless, adequate containment of biological materials is a fundamental program component for both biosafety and biosecurity.

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* In 1996 the United States Hospital Infection Control Practices Advisory Committee introduced a new set of guidelines, "Standard Precautions," to synthesize the major features of Universal Precautions (blood and body fluid) with Body Substance Isolation Precautions (designed to reduce the risk of transmission of pathogens from moist body substances).⁶ Standard Precautions apply to 1) blood; 2) all body fluids, secretions, and excretions except sweat, regardless of whether or not they contain visible blood; 3) non-intact skin; and 4) mucous membranes. For additional information on Standard Precautions, see reference 6 or the CDC Web site: www.cdc.gov.

Section IV—Laboratory Biosafety Level Criteria

The essential elements of the four biosafety levels for activities involving infectious microorganisms and laboratory animals are summarized in Table 2 of this section and discussed in Section 2. The levels are designated in ascending order, by degree of protection provided to personnel, the environment, and the community. Standard microbiological practices are common to all laboratories. Special microbiological practices enhance worker safety, environmental protection, and address the risk of handling agents requiring increasing levels of containment.

Biosafety Level 1

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science.

The following standard practices, safety equipment, and facility requirements apply to BSL-1.

A. *Standard Microbiological Practices*

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:

- a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
 - b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
 - c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
 - d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
 7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
 8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport.
 - a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
 - b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
 9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. The sign may include the name of the agent(s) in use, and the name and phone number of the laboratory supervisor or other responsible personnel. Agent information should be posted in accordance with the institutional policy.
 10. An effective integrated pest management program is required. (See Appendix G.)

11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

B. Special Practices

None required.

C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. Special containment devices or equipment, such as BSCs, are not generally required.
2. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
3. Wear protective eyewear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses in laboratories should also wear eye protection.
4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Wash hands prior to leaving the laboratory. In addition, BSL-1 workers should:
 - a. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
 - b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
 - c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratories should have doors for access control.
2. Laboratories must have a sink for hand washing.
3. The laboratory should be designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
 - a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
 - b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
5. Laboratories windows that open to the exterior should be fitted with screens.

Biosafety Level 2

Biosafety Level 2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that: 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

The following standard and special practices, safety equipment, and facility requirements apply to BSL-2.

A. Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.

4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:
 - a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
 - b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
 - c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
 - d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:
 - a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
 - b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include: the laboratory's biosafety level, the

supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.

10. An effective integrated pest management program is required.
(See Appendix G.)
11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

B. Special Practices

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
2. Laboratory personnel must be provided medical surveillance, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory.
3. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.
4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.
6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.

- a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
 - b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
 9. Animal and plants not associated with the work being performed must not be permitted in the laboratory.
 10. All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.

C. Safety Equipment (*Primary Barriers and Personal Protective Equipment*)

1. Properly maintained BSCs, other appropriate personal protective equipment, or other physical containment devices must be used whenever:
 - a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
 - b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.
2. Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials. Remove protective clothing before leaving for non-laboratory areas, e.g., cafeteria, library, and administrative offices). Dispose of protective clothing appropriately, or deposit it for laundering by the institution. It is recommended that laboratory clothing not be taken home.
3. Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or

decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.

4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should:
 - a. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
 - b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
 - c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
5. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratory doors should be self-closing and have locks in accordance with the institutional policies.
2. Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.
3. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
 - a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
 - b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
5. Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.

6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Vacuum lines should be protected with liquid disinfectant traps.
8. An eyewash station must be readily available.
9. There are no specific requirements for ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.
10. HEPA filtered exhaust air from a Class II BSC can be safely recirculation back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly exhausted to the outside through a hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.
11. A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

Biosafety Level 3

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures.

All procedures involving the manipulation of infectious materials must be conducted within BSCs or other physical containment devices.

A BSL-3 laboratory has special engineering and design features.

The following standard and special safety practices, equipment, and facility requirements apply to BSL-3.

A. *Standard Microbiological Practices*

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.

2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.

Precautions, including those listed below, must always be taken with sharp items. These include:

- a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
 - b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
 - c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
 - d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
 7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
 8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method). Depending on where the decontamination will be performed, the following methods should be used prior to transport:

- a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
 - b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
 10. An effective integrated pest management program is required. (See Appendix G.)
 11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

B. Special Practices

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
2. Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.
3. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.
4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.

5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents.
6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
 - a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
 - b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.
10. All procedures involving the manipulation of infectious materials must be conducted within a BSC, or other physical containment devices. No work with open vessels is conducted on the bench. When a procedure cannot be performed within a BSC, a combination of personal protective equipment and other containment devices, such as a centrifuge safety cup or sealed rotor must be used.

C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. All procedures involving the manipulation of infectious materials must be conducted within a BSC (preferably Class II or Class III), or other physical containment devices.
2. Workers in the laboratory where protective laboratory clothing with a solid-front, such as tie-back or wrap-around gowns, scrub suits, or coveralls. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when contaminated.

3. Eye and face protection (goggles, mask, face shield or other splash guard) is used for anticipated splashes or sprays of infectious or other hazardous materials. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories must also wear eye protection.
4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-3 laboratory workers:
 - a. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
 - b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
 - c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
5. Eye, face, and respiratory protection must be used in rooms containing infected animals.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratory doors must be self-closing and have locks in accordance with the institutional policies. The laboratory must be separated from areas that are open to unrestricted traffic flow within the building. Laboratory access is restricted. Access to the laboratory is through two self-closing doors. A clothing change room (anteroom) may be included in the passageway between the two self-closing doors.
2. Laboratories must have a sink for hand washing. The sink must be hands-free or automatically operated. It should be located near the exit door. If the laboratory is segregated into different laboratories, a sink must also be available for hand washing in each zone. Additional sinks may be required as determined by the risk assessment.
3. The laboratory must be designed so that it can be easily cleaned and decontaminated. Carpets and rugs are not permitted. Seams, floors, walls, and ceiling surfaces should be sealed. Spaces around doors and ventilation openings should be capable of being sealed to facilitate space decontamination.

- a. Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Consideration should be given to the installation of seamless, sealed, resilient or poured floors, with integral cove bases.
- b. Walls should be constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.
- c. Ceilings should be constructed, sealed, and finished in the same general manner as walls.

Decontamination of the entire laboratory should be considered when there has been gross contamination of the space, significant changes in laboratory usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the laboratory must be based on the risk assessment.

4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning.
 - a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
 - b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
5. All windows in the laboratory must be sealed.
6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
8. An eyewash station must be readily available in the laboratory.
9. A ducted air ventilation system is required. This system must provide sustained directional airflow by drawing air into the laboratory from “clean” areas toward “potentially contaminated” areas. The laboratory shall be designed such that under failure conditions the airflow will not be reversed.
 - a. Laboratory personnel must be able to verify directional airflow. A visual monitoring device, which confirms directional airflow, must be provided at the laboratory entry. Audible alarms should be considered to notify personnel of air flow disruption.

- b. The laboratory exhaust air must not re-circulate to any other area of the building.
- c. The laboratory building exhaust air should be dispersed away from occupied areas and from building air intake locations or the exhaust air must be HEPA filtered.

HEPA filter housings should have gas-tight isolation dampers, decontamination ports, and/or bag-in/bag-out (with appropriate decontamination procedures) capability. The HEPA filter housing should allow for leak testing of each filter and assembly. The filters and the housing should be certified at least annually.

- 10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly exhausted to the outside through a hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be certified at least annually to assure correct performance. Class III BSCs must be directly (hard) connected up through the second exhaust HEPA filter of the cabinet. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.
- 11. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, or other validated decontamination method).
- 12. Equipment that may produce infectious aerosols must be contained in primary barrier devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters should be tested and/or replaced at least annually.
- 13. Facility design consideration should be given to means of decontaminating large pieces of equipment before removal from the laboratory.
- 14. Enhanced environmental and personal protection may be required by the agent summary statement, risk assessment, or applicable local, state, or federal regulations. These laboratory enhancements may include, for example, one or more of the following: an anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities; gas tight dampers to facilitate laboratory isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; and advanced access control devices, such as biometrics.

15. The BSL-3 facility design, operational parameters, and procedures must be verified and documented prior to operation. Facilities must be re-verified and documented at least annually.

Biosafety Level 4

Biosafety Level 4 is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments, or a related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring BSL-4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level, or re-designate the level. Laboratory staff must have specific and thorough training in handling extremely hazardous infectious agents. Laboratory staff must understand the primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics. All laboratory staff and supervisors must be competent in handling agents and procedures requiring BSL-4 containment. The laboratory supervisor in accordance with institutional policies controls access to the laboratory.

There are two models for BSL-4 laboratories:

1. A *Cabinet Laboratory*—Manipulation of agents must be performed in a Class III BSC; and
2. A *Suit Laboratory*—Personnel must wear a positive pressure supplied air protective suit.

BSL-4 cabinet and suit laboratories have special engineering and design features to prevent microorganisms from being disseminated into the environment.

The following standard and special safety practices, equipment, and facilities apply to BSL-4.

A. Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
3. Mechanical pipetting devices must be used.
4. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented.

Precautions, including those listed below, must be taken with any sharp items. These include:

- a. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
 - b. Use of needles and syringes or other sharp instruments should be restricted in the laboratory, except when there is no practical alternative.
 - c. Used needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal or decontamination. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal, located as close to the point of use as possible.
 - d. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.
5. Perform all procedures to minimize the creation of splashes and/or aerosols.
 6. Decontaminate work surfaces with appropriate disinfectant after completion of work and after any spill or splash of potentially infectious material.
 7. Decontaminate all wastes before removal from the laboratory by an effective and validated method.
 8. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
 9. An effective integrated pest management program is required. (See Appendix G.)
 10. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information

regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

B. Special Practices

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry requirements in accordance with institutional policies.

Only persons whose presence in the facility or individual laboratory rooms is required for scientific or support purposes are authorized to enter.

Entry into the facility must be limited by means of secure, locked doors. A logbook, or other means of documenting the date and time of all persons entering and leaving the laboratory must be maintained.

While the laboratory is operational, personnel must enter and exit the laboratory through the clothing change and shower rooms except during emergencies. All personal clothing must be removed in the outer clothing change room. All persons entering the laboratory must use laboratory clothing, including undergarments, pants, shirts, jumpsuits, shoes, and gloves (as appropriate). All persons leaving the laboratory must take a personal body shower. Used laboratory clothing must not be removed from the inner change room through the personal shower. These items must be treated as contaminated materials and decontaminated before laundering.

After the laboratory has been completely decontaminated and all infectious agents are secured, necessary staff may enter and exit without following the clothing change and shower requirements described above.

2. Laboratory personnel and support staff must be provided appropriate occupational medical services including medical surveillance and available immunizations for agents handled or potentially present in the laboratory. A system must be established for reporting and documenting laboratory accidents, exposures, employee absenteeism and for the medical surveillance of potential laboratory-associated illnesses. An essential adjunct to such an occupational medical services system is the availability of a facility for the isolation and medical care of personnel with potential or known laboratory-acquired infections.
3. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.

4. A laboratory-specific biosafety manual must be prepared. The biosafety manual must be available, accessible, and followed.
5. The laboratory supervisor is responsible for ensuring that laboratory personnel:
 - a. Demonstrate high proficiency in standard and special microbiological practices, and techniques for working with agents requiring BSL-4 containment.
 - b. Receive appropriate training in the practices and operations specific to the laboratory facility.
 - c. Receive annual updates and additional training when procedural or policy changes occur.
6. Removal of biological materials that are to remain in a viable or intact state from the laboratory must be transferred to a non-breakable, sealed primary container and then enclosed in a non-breakable, sealed secondary container. These materials must be transferred through a disinfectant dunk tank, fumigation chamber, or decontamination shower. Once removed, packaged viable material must not be opened outside BSL-4 containment unless inactivated by a validated method.
7. Laboratory equipment must be routinely decontaminated, as well as after spills, splashes, or other potential contamination.
 - a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by appropriate professional staff, or others properly trained and equipped to work with infectious material. A spill procedure must be developed and posted within the laboratory.
 - b. Equipment must be decontaminated using an effective and validated method before repair, maintenance, or removal from the laboratory. The interior of the Class III cabinet as well as all contaminated plenums, fans and filters must be decontaminated using a validated gaseous or vapor method.
 - c. Equipment or material that might be damaged by high temperatures or steam must be decontaminated using an effective and validated procedure such as a gaseous or vapor method in an airlock or chamber designed for this purpose.
8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All incidents must be reported to the laboratory supervisor, institutional management and appropriate

laboratory personnel as defined in the laboratory biosafety manual. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.

9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.
10. Supplies and materials that are not brought into the BSL-4 laboratory through the change room, must be brought in through a previously decontaminated double-door autoclave, fumigation chamber, or airlock. After securing the outer doors, personnel within the laboratory retrieve the materials by opening the interior doors of the autoclave, fumigation chamber, or airlock. These doors must be secured after materials are brought into the facility. The doors of the autoclave or fumigation chamber are interlocked in a manner that prevents opening of the outer door unless the autoclave or fumigation chamber has been operated through a decontamination cycle.

Only necessary equipment and supplies should be stored inside the BSL-4 laboratory. All equipment and supplies taken inside the laboratory must be decontaminated before removal from the laboratory.

11. Daily inspections of essential containment and life support systems must be completed and documented before laboratory work is initiated to ensure that the laboratory is operating according to established parameters.
12. Practical and effective protocols for emergency situations must be established. These protocols must include plans for medical emergencies, facility malfunctions, fires, escape of animals within the laboratory, and other potential emergencies. Training in emergency response procedures must be provided to emergency response personnel and other responsible staff according to institutional policies.

C. Safety Equipment (*Primary Barriers and Personal Protective Equipment*)

Cabinet Laboratory

1. All manipulations of infectious materials within the laboratory must be conducted in the Class III biological safety cabinet.

Double-door, pass through autoclaves must be provided for decontaminating materials passing out of the Class III BSC(s). The autoclave doors must be interlocked so that only one can be opened at any time and be automatically controlled so that the outside door to the autoclave can only be opened after the decontamination cycle has been completed.

The Class III cabinet must also have a pass-through dunk tank, fumigation chamber, or equivalent decontamination method so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the cabinet. Containment must be maintained at all times.

The Class III cabinet must have a HEPA filter on the supply air intake and two HEPA filters in series on the exhaust outlet of the unit. There must be gas tight dampers on the supply and exhaust ducts of the cabinet to permit gas or vapor decontamination of the unit. Ports for injection of test medium must be present on all HEPA filter housings.

The interior of the Class III cabinet must be constructed with smooth finishes that can be easily cleaned and decontaminated. All sharp edges on cabinet finishes must be eliminated to reduce the potential for cuts and tears of gloves. Equipment to be placed in the Class III cabinet should also be free of sharp edges or other surfaces that may damage or puncture the cabinet gloves.

Class III cabinet gloves must be inspected for damage prior to use and changed if necessary. Gloves should be replaced annually during cabinet re-certification.

The cabinet should be designed to permit maintenance and repairs of cabinet mechanical systems (refrigeration, incubators, centrifuges, etc.) to be performed from the exterior of the cabinet whenever possible.

Manipulation of high concentrations or large volumes of infectious agents within the Class III cabinet should be performed using physical containment devices inside the cabinet whenever practical. Such materials should be centrifuged inside the cabinet using sealed rotor heads or centrifuge safety cups.

The Class III cabinet must be certified at least annually.

2. Workers in the laboratory must wear protective laboratory clothing with a solid-front, such as tie-back or wrap-around gowns, scrub suits, or coveralls. No personal clothing, jewelry, or other items except eyeglasses should be taken past the personal shower area. All protective clothing must be removed in the dirty side change room before showering. Reusable clothing must be autoclaved prior to removal from the laboratory for laundering.
3. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment. Prescription eyeglasses must be decontaminated before removal through the personal body shower.

4. Disposable gloves must be worn underneath cabinet gloves to protect the worker from exposure should a break or tear occur in a cabinet glove. Gloves must not be worn outside the laboratory. Alternatives to latex gloves should be available. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste.

Suit Laboratory

1. All procedures must be conducted by personnel wearing a one-piece positive pressure supplied air suit.

All manipulations of infectious agents must be performed within a BSC or other primary barrier system.

Equipment that may produce aerosols must be contained in primary barrier devices that exhaust air through HEPA filtration before being discharged into the laboratory. These HEPA filters should be tested annually and replaced as needed.

HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's specifications.

2. Workers must wear laboratory clothing, such as scrub suits, before entering the room used for donning positive pressure suits. All laboratory clothing must be removed in the dirty side change room before entering the personal shower.
3. Inner disposable gloves must be worn to protect against break or tears in the outer suit gloves. Disposable gloves must not be worn outside the change area. Alternatives to latex gloves should be available. Do not wash or reuse disposable gloves. Inner gloves must be removed and discarded in the inner change room prior to entering the personal shower. Dispose of used gloves with other contaminated waste.
4. Decontamination of outer suit gloves is performed during laboratory operations to remove gross contamination and minimize further contamination of the laboratory.

D. Laboratory Facilities (Secondary Barriers)

Cabinet Laboratory

1. The BSL-4 cabinet laboratory consists of either a separate building or a clearly demarcated and isolated zone within a building. Laboratory doors must have locks in accordance with the institutional policies.

Rooms in the facility must be arranged to ensure sequential passage through an inner (dirty) changing area, a personal shower and an outer (clean) change room upon exiting the room(s) containing the Class III BSC(s).

An automatically activated emergency power source must be provided at a minimum for the laboratory exhaust system, life support systems, alarms, lighting, entry and exit controls, BSCs, and door gaskets. Monitoring and control systems for air supply, exhaust, life support, alarms, entry and exit controls, and security systems should be on an uninterrupted power supply (UPS).

A double-door autoclave, dunk tank, fumigation chamber, or ventilated airlock must be provided at the containment barrier for the passage of materials, supplies, or equipment.

2. A hands-free sink must be provided near the door of the cabinet room(s) and the inner change room. A sink must be provided in the outer change room. All sinks in the room(s) containing the Class III BSC must be connected to the wastewater decontamination system.
3. Walls, floors, and ceilings of the laboratory must be constructed to form a sealed internal shell to facilitate fumigation and prohibit animal and insect intrusion. The internal surfaces of this shell must be resistant to chemicals used for cleaning and decontamination of the area. Floors must be monolithic, sealed and coved.

All penetrations in the internal shell of the laboratory and inner change room must be sealed.

Openings around doors into the cabinet room and inner change room must be minimized and capable of being sealed to facilitate decontamination.

Drains in the laboratory floor (if present) must be connected directly to the liquid waste decontamination system.

Services and plumbing that penetrate the laboratory walls, floors, or ceiling must be installed to ensure that no backflow from the laboratory occurs. These penetrations must be fitted with two (in series) backflow prevention devices. Consideration should be given to locating these devices outside of containment. Atmospheric venting systems must be provided with two HEPA filters in series and be sealed up to the second filter.

Decontamination of the entire cabinet must be performed using a validated gaseous or vapor method when there have been significant changes in cabinet usage, before major renovations or maintenance shut downs, and in other situations, as determined by risk assessment.

Selection of the appropriate materials and methods used for decontamination must be based on the risk assessment.

4. Laboratory furniture must be of simple construction, capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning and decontamination. Chairs and other furniture must be covered with a non-porous material that can be easily decontaminated.
5. Windows must be break-resistant and sealed.
6. If Class II BSCs are needed in the cabinet laboratory, they must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. Class II cabinets should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Central vacuum systems are not recommended. If, however, there is a central vacuum system, it must not serve areas outside the cabinet room. Two in-line HEPA filters must be placed near each use point. Filters must be installed to permit in-place decontamination and replacement.
8. An eyewash station must be readily available in the laboratory.
9. A dedicated non-recirculating ventilation system is provided. Only laboratories with the same HVAC requirements (i.e., other BSL-4 labs, ABSL-4, BSL-3-Ag labs) may share ventilation systems if gas-tight dampers and HEPA filters isolate each individual laboratory system.

The supply and exhaust components of the ventilation system must be designed to maintain the laboratory at negative pressure to surrounding areas and provide differential pressure or directional airflow, as appropriate, between adjacent areas within the laboratory.

Redundant supply fans are recommended. Redundant exhaust fans are required. Supply and exhaust fans must be interlocked to prevent positive pressurization of the laboratory.

The ventilation system must be monitored and alarmed to indicate malfunction or deviation from design parameters. A visual monitoring device must be installed near the clean change room so proper differential pressures within the laboratory may be verified prior to entry.

Supply air to and exhaust air from the cabinet room, inner change room, and fumigation/decontamination chambers must pass through HEPA filter(s). The air exhaust discharge must be located away from occupied spaces and building air intakes.

All HEPA filters should be located as near as practicable to the cabinet and laboratory in order to minimize the length of potentially contaminated ductwork. All HEPA filters must be tested and certified annually.

The HEPA filter housings should be designed to allow for *in situ* decontamination and validation of the filter prior to removal. The design of the HEPA filter housing must have gas-tight isolation dampers, decontamination ports, and ability to scan each filter assembly for leaks.

10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to the manufacturer's recommendations. If BSC exhaust is to be recirculated to the outside, BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a hard ducted, direct connection ensuring that cabinet exhaust air passes through two (2) HEPA filters—including the HEPA in the BSC—prior to release outside. Provisions to assure proper safety cabinet performance and air system operation must be verified.

Class III BSCs must be directly and independently exhausted through two HEPA filters in series. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.

11. Pass through dunk tanks, fumigation chambers, or equivalent decontamination methods must be provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the cabinet room(s). Access to the exit side of the pass-through shall be limited to those individuals authorized to be in the BSL-4 laboratory.
12. Liquid effluents from cabinet room sinks, floor drains, autoclave chambers, and other sources within the cabinet room must be decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer.

Decontamination of all liquid wastes must be documented. The decontamination process for liquid wastes must be validated physically and biologically. Biological validation must be performed annually or more often if required by institutional policy.

Effluents from showers and toilets may be discharged to the sanitary sewer without treatment.

13. A double-door, pass through autoclave(s) must be provided for decontaminating materials passing out of the cabinet laboratory. Autoclaves that open outside of the laboratory must be sealed to the interior wall. This bioseal must be durable and airtight and capable of

expansion and contraction. Positioning the bioseal so that the equipment can be accessed and maintained from outside the laboratory is strongly recommended. The autoclave doors must be interlocked so that only one can be opened at any time and be automatically controlled so that the outside door to the autoclave can only be opened after the decontamination cycle has been completed.

Gas and liquid discharge from the autoclave chamber must be decontaminated. When feasible, autoclave decontamination processes should be designed so that unfiltered air or steam exposed to infectious material cannot be released to the environment.

14. The BSL-4 facility design parameters and operational procedures must be documented. The facility must be tested to verify that the design and operational parameters have been met prior to operation. Facilities must also be re-verified annually. Verification criteria should be modified as necessary by operational experience.
15. Appropriate communication systems must be provided between the laboratory and the outside (e.g., voice, fax, and computer). Provisions for emergency communication and emergency access or egress must be developed and implemented.

Suit Laboratory

1. The BSL-4 suit laboratory consists of either a separate building or a clearly demarcated and isolated zone within a building. Laboratory doors must have locks in accordance with the institutional policies.

Rooms in the facility must be arranged to ensure exit by sequential passage through the chemical shower, inner (dirty) change room, personal shower, and outer (clean) changing area.

Entry into the BSL-4 laboratory must be through an airlock fitted with airtight doors. Personnel who enter this area must wear a positive pressure suit supplied with HEPA filtered breathing air. The breathing air systems must have redundant compressors, failure alarms and emergency backup.

A chemical shower must be provided to decontaminate the surface of the positive pressure suit before the worker leaves the laboratory. In the event of an emergency exit or failure of the chemical shower system, a method for decontaminating positive pressure suits, such as a gravity fed supply of chemical disinfectant, is needed.

An automatically activated emergency power source must be provided, at a minimum, for the laboratory exhaust system, life support systems, alarms, lighting, entry and exit controls, BSCs, and door gaskets.

Monitoring and control systems for air supply, exhaust, life support, alarms, entry and exit controls, and security systems should be on a UPS.

A double-door autoclave, dunk tank, or fumigation chamber must be provided at the containment barrier for the passage of materials, supplies, or equipment in or out of the laboratory.

2. Sinks inside the suit laboratory should be placed near procedure areas and be connected to the wastewater decontamination system.
3. Walls, floors, and ceilings of the laboratory must be constructed to form a sealed internal shell to facilitate fumigation and prohibit animal and insect intrusion. The internal surfaces of this shell must be resistant to chemicals used for cleaning and decontamination of the area. Floors must be monolithic, sealed and coved.

All penetrations in the internal shell of the laboratory, suit storage room and the inner change room must be sealed.

Drains, if present, in the laboratory floor must be connected directly to the liquid waste decontamination system. Sewer vents must have protection against insect and animal intrusion.

Services and plumbing that penetrate the laboratory walls, floors, or ceiling must be installed to ensure that no backflow from the laboratory occurs. These penetrations must be fitted with two (in series) backflow prevention devices. Consideration should be given to locating these devices outside of containment. Atmospheric venting systems must be provided with two HEPA filters in series and be sealed up to the second filter.

4. Laboratory furniture must be of simple construction, capable of supporting anticipated loading and uses. Sharp edges and corners should be avoided. Spaces between benches, cabinets, and equipment must be accessible for cleaning and decontamination. Chairs and other furniture must be covered with a non-porous material that can be easily decontaminated.
5. Windows must be break-resistant and sealed.
6. BSCs and other primary containment barrier systems must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Central vacuum systems are not recommended. If, however, there is a central vacuum system, it must not serve areas outside the BSL-4 laboratory. Two in-line HEPA filters must be placed near each use point. Filters must be installed to permit in-place decontamination and replacement.

8. An eyewash station must be readily available in the laboratory area for use during maintenance and repair activities.
9. A dedicated, non-recirculating ventilation system is provided. Only laboratories with the same HVAC requirements (i.e., other BSL-4 labs, ABSL-4, BSL-3 Ag labs) may share ventilation systems if gas-tight dampers and HEPA filters isolate each individual laboratory system.

The supply and exhaust components of the ventilation system must be designed to maintain the laboratory at negative pressure to surrounding areas and provide differential pressure or directional airflow as appropriate between adjacent areas within the laboratory.

Redundant supply fans are recommended. Redundant exhaust fans are required. Supply and exhaust fans must be interlocked to prevent positive pressurization of the laboratory.

The ventilation system must be monitored and alarmed to indicate malfunction or deviation from design parameters. A visual monitoring device must be installed near the clean change room so proper differential pressures within the laboratory may be verified prior to entry.

Supply air to the laboratory, including the decontamination shower, must pass through a HEPA filter. All exhaust air from the suit laboratory, decontamination shower and fumigation or decontamination chambers must pass through two HEPA filters, in series, before discharge to the outside. The exhaust air discharge must be located away from occupied spaces and air intakes.

All HEPA filters must be located as near as practicable to the laboratory in order to minimize the length of potentially contaminated ductwork. All HEPA filters must be tested and certified annually.

The HEPA filter housings must be designed to allow for *in situ* decontamination and validation of the filter prior to removal. The design of the HEPA filter housing must have gas-tight isolation dampers, decontamination ports, and ability to scan each filter assembly for leaks.

10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to the manufacturer's recommendations. Biological safety cabinets can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.

11. Pass through dunk tanks, fumigation chambers, or equivalent decontamination methods must be provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the BSL-4 laboratory. Access to the exit side of the pass-through shall be limited to those individuals authorized to be in the BSL-4 laboratory.
12. Liquid effluents from chemical showers, sinks, floor drains, autoclave chambers, and other sources within the laboratory must be decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer.

Decontamination of all liquid wastes must be documented. The decontamination process for liquid wastes must be validated physically and biologically. Biological validation must be performed annually or more often if required by institutional policy.

Effluents from personal body showers and toilets may be discharged to the sanitary sewer without treatment.

13. A double-door, pass through autoclave(s) must be provided for decontaminating materials passing out of the cabinet laboratory. Autoclaves that open outside of the laboratory must be sealed to the interior wall. This bioseal must be durable, airtight, and capable of expansion and contraction. Positioning the bioseal so that the equipment can be accessed and maintained from outside the laboratory is strongly recommended. The autoclave doors must be interlocked so that only one can be opened at any time and be automatically controlled so that the outside door to the autoclave can only be opened after the decontamination cycle has been completed.

Gas and liquid discharge from the autoclave chamber must be decontaminated. When feasible, autoclave decontamination processes should be designed so that unfiltered air or steam exposed to infectious material cannot be released to the environment.
14. The BSL-4 facility design parameters and operational procedures must be documented. The facility must be tested to verify that the design and operational parameters have been met prior to operation. Facilities must also be re-verified annually. Verification criteria should be modified as necessary by operational experience.
15. Appropriate communication systems must be provided between the laboratory and the outside (e.g., voice, fax, and computer). Provisions for emergency communication and emergency access or egress must be developed and implemented.

Table 2. Summary of Recommended Biosafety Levels for Infectious Agents

BSL	Agents	Practices	Primary Barriers and Safety Equipment	Facilities (Secondary Barriers)
1	Not known to consistently cause diseases in healthy adults	Standard microbiological practices	<ul style="list-style-type: none"> ■ No primary barriers required. ■ PPE: laboratory coats and gloves; eye, face protection, as needed 	Laboratory bench and sink required
2	<ul style="list-style-type: none"> ■ Agents associated with human disease ■ Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure 	BSL-1 practice plus: <ul style="list-style-type: none"> ■ Limited access ■ Biohazard warning signs ■ "Sharps" precautions ■ Biosafety manual defining any needed waste decontamination or medical surveillance policies 	Primary barriers: <ul style="list-style-type: none"> ■ BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials ■ PPE: Laboratory coats, gloves, face and eye protection, as needed 	BSL-1 plus: <ul style="list-style-type: none"> ■ Autoclave available
3	Indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure	BSL-2 practice plus: <ul style="list-style-type: none"> ■ Controlled access ■ Decontamination of all waste ■ Decontamination of laboratory clothing before laundering 	Primary barriers: <ul style="list-style-type: none"> ■ BSCs or other physical containment devices used for all open manipulations of agents ■ PPE: Protective laboratory clothing, gloves, face, eye and respiratory protection, as needed 	BSL-2 plus: <ul style="list-style-type: none"> ■ Physical separation from access corridors ■ Self-closing, double-door access ■ Exhausted air not recirculated ■ Negative airflow into laboratory ■ Entry through airlock or anteroom ■ Hand washing sink near laboratory exit
4	<ul style="list-style-type: none"> ■ Dangerous/exotic agents which post high individual risk of aerosol-transmitted laboratory infections that are frequently fatal, for which there are no vaccines or treatments ■ Agents with a close or identical antigenic relationship to an agent requiring BSL-4 until data are available to redesignate the level ■ Related agents with unknown risk of transmission 	BSL-3 practices plus: <ul style="list-style-type: none"> ■ Clothing change before entering ■ Shower on exit ■ All material decontaminated on exit from facility 	Primary barriers: <ul style="list-style-type: none"> ■ All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure suit 	BSL-3 plus: <ul style="list-style-type: none"> ■ Separate building or isolated zone ■ Dedicated supply and exhaust, vacuum, and decontamination systems ■ Other requirements outlined in the text

Section V—Vertebrate Animal Biosafety Level Criteria for Vivarium Research Facilities

This guidance is provided for the use of experimentally infected animals housed in indoor research facilities (e.g., vivaria), and is also useful in the maintenance of laboratory animals that may naturally harbor zoonotic infectious agents. In both instances, the institutional management must provide facilities, staff, and established practices that reasonably ensure appropriate levels of environmental quality, safety, security and care for the laboratory animal. Laboratory animal facilities are a special type of laboratory. As a general principle, the biosafety level (facilities, practices, and operational requirements) recommended for working with infectious agents *in vivo* and *in vitro* are comparable.

The animal room can present unique problems. In the animal room, the activities of the animals themselves can present unique hazards not found in standard microbiological laboratories. Animals may generate aerosols, they may bite and scratch, and they may be infected with a zoonotic agent. The co-application of Biosafety Levels and the Animal Biosafety Levels are determined by a protocol-driven risk assessment.

These recommendations presuppose that laboratory animal facilities, operational practices, and quality of animal care meet applicable standards and regulations (e.g., *Guide for the Care and Use of Laboratory Animals*¹ and *Laboratory Animal Welfare Regulations*²) and that appropriate species have been selected for animal experiments. In addition, the organization must have an occupational health and safety program that addresses potential hazards associated with the conduct of laboratory animal research. The following publication by the Institute for Laboratory Animal Research (ILAR), *Occupational Health and Safety in the Care and Use of Research Animals*³, is most helpful in this regard. Additional safety guidance on working with non-human primates is available in the ILAR publication, *Occupational Health and Safety in the Care and Use of Nonhuman Primates*.⁴

Facilities for laboratory animals used in studies of infectious or non-infectious disease should be physically separate from other activities such as animal production and quarantine, clinical laboratories, and especially from facilities providing patient care. Traffic flow that will minimize the risk of cross contamination should be incorporated into the facility design.

The recommendations detailed below describe four combinations of practices, safety equipment, and facilities for experiments with animals involved in infectious disease research and other studies that may require containment. These four combinations, designated Animal Biosafety Levels (ABSL) 1-4, provide increasing levels of protection to personnel and to the environment, and are recommended as minimal standards for activities involving infected laboratory animals. The four ABSLs describe animal facilities and practices

applicable to work with animals infected with agents assigned to Biosafety Levels 1-4, respectively. Investigators that are inexperienced in conducting these types of experiments should seek help in designing their experiments from individuals who are experienced in this special work.

In addition to the animal biosafety levels described in this section, the USDA has developed facility parameters and work practices for handling agents of agriculture significance. Appendix D includes a discussion on Animal Biosafety Level 3 Agriculture (BSL-3-Ag). USDA requirements are unique to agriculture because of the necessity to protect the environment from pathogens of economic or environmental impact. Appendix D also describes some of the enhancements beyond BSL/ABSL-3 that may be required by USDA-APHIS when working in the laboratory or vivarium with certain veterinary agents of concern.

Facility standards and practices for invertebrate vectors and hosts are not specifically addressed in this section. The reader is referred to Appendix E for more information on the Arthropod Containment Guidelines.

Animal Biosafety Level 1

Animal Biosafety Level 1 is suitable for work in animals involving well-characterized agents that are not known to cause disease in immunocompetent adult humans, and present minimal potential hazard to personnel and the environment.

ABSL-1 facilities should be separated from the general traffic patterns of the building and restricted as appropriate. Special containment equipment or facility design may be required as determined by appropriate risk assessment. (See Section 2, Biological Risk Assessment.)

Personnel must have specific training in animal facility procedures and must be supervised by an individual with adequate knowledge of potential hazards and experimental animal procedures.

The following standard practices, safety equipment, and facility requirements apply to ABSL-1.

A. *Standard Microbiological Practices*

1. The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergencies.

Each institute must assure that worker safety and health concerns are addressed as part of the animal protocol review.

Prior to beginning a study animal protocols must also be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC)⁵ and the Institutional Biosafety Committee.

2. A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety professionals. The safety manual must be available and accessible. Personnel are advised of potential hazards and are required to read and follow instructions on practices and procedures.
3. The supervisor must ensure that animal care, laboratory and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to prevent exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates and additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.
4. An appropriate medical surveillance program is in place, as determined by risk assessment. The need for an animal allergy prevention program should be considered.

Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care and manipulations.

Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women of childbearing age should be provided information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

Personnel using respirators must be enrolled in an appropriately constituted respiratory protection program.

5. A sign incorporating safety information must be posted at the entrance to the areas where infectious materials and/or animals are housed or are manipulated. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of specific infectious agents is recommended when more than one agent is being used within an animal room.

Security-sensitive agent information should be posted in accordance with the institutional policy.

Advance consideration should be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.^{1,3,4}

6. Access to the animal room is limited. Only those persons required for program or support purposes are authorized to enter the facility.

All persons including facility personnel, service workers, and visitors are advised of the potential hazards (natural or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.

7. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.

Gloves are worn to prevent skin contact with contaminated, infectious and hazardous materials, and when handling animals.

Gloves and personal protective equipment should be removed in a manner that minimizes transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated.

Persons must wash their hands after removing gloves, and before leaving the areas where infectious materials and/or animals are housed or are manipulated.

Eye and face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.

8. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside of the laboratory in cabinets or refrigerators designed and used for this purpose.
9. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
10. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.
11. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented.

When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:

- a. Use of needles and syringes or other sharp instruments in the animal facility is limited to situations where there is no alternative

for such procedures as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.

- b. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the work site as possible.
 - c. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
 - d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
 - e. Equipment containing sharp edges and corners should be avoided.
12. Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent, and after any spills, splashes, or other overt contamination.
 13. Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/ or animals are housed or are manipulated.
 14. An effective integrated pest management program is required.
(See Appendix G.)
 15. All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof, covered containers for appropriate disposal in compliance with applicable institutional, local and state requirements.

Decontaminate all potentially infectious materials before disposal using an effective method.

B. Special Practices

None required.

C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. A risk assessment should determine the appropriate type of personal protective equipment to be utilized.

2. Special containment devices or equipment may not be required as determined by appropriate risk assessment.
3. Protective laboratory coats, gowns, or uniforms may be required to prevent contamination of personal clothing.

Protective outer clothing is not worn outside areas where infectious materials and/or animals are housed or manipulated. Gowns and uniforms are not worn outside the facility.

4. Protective eyewear is worn when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates.

Persons having contact with NHPs must assess risk of mucous membrane exposure and wear protective equipment (e.g., masks, goggles, face shields, etc.) as appropriate for the task to be performed.

5. Gloves are worn to protect hands from exposure to hazardous materials.

A risk assessment should be performed to identify the appropriate glove for the task and alternatives to latex gloves should be available.

Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.

Gloves must not be worn outside the animal rooms.

Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials.

Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste.

6. Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Hand washing should occur after the removal of gloves.

D. Laboratory Facilities (Secondary Barriers)

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking.

Access to the animal facility is restricted.

Doors to areas where infectious materials and/or animals are housed, open inward, are self-closing, are kept closed when experimental animals are present, and should never be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.

2. The animal facility must have a sink for hand washing.

Sink traps are filled with water, and/or appropriate liquid to prevent the migration of vermin and gases.

3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant. Floors must be slip resistant, impervious to liquids, and resistant to chemicals.

It is recommended that penetrations in floors, walls and ceiling surfaces be sealed, including openings around ducts, doors and doorframes, to facilitate pest control and proper cleaning.

4. Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

Chairs used in animal area must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Sharp edges and corners should be avoided.

5. External windows are not recommended; if present windows must be resistant to breakage. Where possible, windows should be sealed. If the animal facility has windows that open, they are fitted with fly screens. The presence of windows may impact facility security and therefore should be assessed by security personnel.

6. Ventilation should be provided in accordance with the *Guide for Care and Use of Laboratory Animals*.¹ No recirculation of exhaust air may occur. It is recommended that animal rooms have inward directional airflow.

Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.

7. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.
8. If floor drains are provided, the traps are filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.

9. Cages are washed manually or preferably in a mechanical cage washer. The mechanical cage washer should have a final rinse temperature of at least 180°F. If manual cage washing is utilized, ensure that appropriate disinfectants are selected.
10. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
11. Emergency eyewash and shower are readily available; location is determined by risk assessment.

Animal Biosafety Level 2

Animal Biosafety Level 2 builds upon the practices, procedures, containment equipment, and facility requirements of ABSL-1. ABSL-2 is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment. It also addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure.

ABSL-2 requires that: 1) access to the animal facility is restricted; 2) personnel must have specific training in animal facility procedures, the handling of infected animals and the manipulation of pathogenic agents; 3) personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations and husbandry procedures; and 4) BSCs or other physical containment equipment is used when procedures involve the manipulation of infectious materials, or where aerosols or splashes may be created.

Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment. Implementation of employee occupational health programs should be considered.

The following standard and special practices, safety equipment, and facility requirements apply to ABSL-2:

A. *Standard Microbiological Practices*

1. The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergencies.

Each organization must assure that worker safety and health concerns are addressed as part of the animal protocol review.

Prior to beginning a study, animal protocols must also be reviewed and approved by the IACUC⁵ and the Institutional Biosafety Committee.

2. A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety professionals.

The safety manual must be available and accessible. Personnel are advised of potential hazards, and are required to read and follow instructions on practices and procedures.

Consideration should be given to specific biohazards unique to the animal species and protocol in use.

3. The supervisor must ensure that animal care, laboratory, and support personnel receive appropriate training regarding their duties, animal husbandry procedure, potential hazards, manipulations of infectious agents, necessary precautions to prevent hazard or exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates or additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.
4. An appropriate medical surveillance program is in place, as determined by risk assessment. The need for an animal allergy prevention program should be considered.

Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care and manipulations.

Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women of childbearing age should be provided information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

Personnel using respirators must be enrolled in an appropriately constituted respiratory protection program.

5. A sign incorporating the universal biohazard symbol must be posted at the entrance to areas where infectious materials and/ or animals are housed or are manipulated when infectious agents are present. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor's name (or names of other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of all infectious agents is necessary when more than one agent is being used within an animal room.

Security-sensitive agent information and occupational health requirements should be posted in accordance with the institutional policy.

Advance consideration should be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.^{1,3,4}

6. Access to the animal room is limited. Only those persons required for program or support purposes are authorized to enter the animal facility and the areas where infectious materials and/or animals are housed or manipulated.

All persons including facility personnel, service workers, and visitors are advised of the potential hazards (physical, naturally occurring, or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.

7. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.

Gloves are worn to prevent skin contact with contaminated, infectious and hazardous materials and when handling animals.

Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated.

Persons must wash their hands after removing gloves, and before leaving the areas where infectious materials and/or animals are housed or are manipulated.

Eye, face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.

8. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside of the laboratory in cabinets or refrigerators designated and used for this purpose.
9. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
10. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.
11. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions must always be taken with sharp items. These include:

- a. The use of needles and syringes or other sharp instruments in the animal facility is limited to situations where there is no alternative such as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
 - b. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used, disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the work site as possible.
 - c. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
 - d. Broken glassware must not be handled directly; it should be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
 - e. Use of equipment with sharp edges and corners should be avoided.
12. Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent, and after any spills, splashes, or other overt contamination.
 13. Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/ or animals are housed or manipulated.
 14. An effective integrated pest management program is required.
(See Appendix G.)
 15. All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof containers for appropriate disposal in compliance with applicable institutional, local and state requirements.

Decontaminate all potentially infectious materials before disposal using an effective method.

B. Special Practices

1. Animal care staff, laboratory and routine support personnel must be provided a medical surveillance program as dictated by the risk assessment and administered appropriate immunizations for agents handled or potentially present, before entry into animal rooms.

When appropriate, a base line serum sample should be stored.

2. Procedures involving a high potential for generating aerosols should be conducted within a biosafety cabinet or other physical containment device. When a procedure cannot be performed within a biosafety cabinet, a combination of personal protective equipment and other containment devices must be used.

Restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint devices, chemical restraint medications) should be used whenever possible.

3. Decontamination by an appropriate method (e.g. autoclave, chemical disinfection, or other approved decontamination methods) is necessary for all potentially infectious materials and animal waste before movement outside the areas where infectious materials and/or animals are housed or are manipulated. This includes potentially infectious animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse.

A method for decontaminating routine husbandry equipment, sensitive electronic and medical equipment should be identified and implemented.

Materials to be decontaminated outside of the immediate areas where infectious materials and/or animals are housed or are manipulated must be placed in a durable, leak proof, covered container and secured for transport. The outer surface of the container is disinfected prior to moving materials. The transport container must have a universal biohazard label.

Develop and implement an appropriate waste disposal program in compliance with applicable institutional, local and state requirements. Autoclaving of content prior to incineration is recommended.

4. Equipment, cages, and racks should be handled in a manner that minimizes contamination of other areas.

Equipment must be decontaminated before repair, maintenance, or removal from the areas where infectious materials and/or animals are housed or are manipulated.

5. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
6. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the safety manual. All such incidents must be reported to the animal facility supervisor or personnel designated by the institution. Medical evaluation, surveillance, and treatment should be provided as appropriate and records maintained.

C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. Properly maintained BSCs, personal protective equipment (e.g., gloves, lab coats, face shields, respirators, etc.) and/or other physical containment devices or equipment, are used whenever conducting procedures with a potential for creating aerosols, splashes, or other potential exposures to hazardous materials. These include necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals.

When indicated by risk assessment, animals are housed in primary biosafety containment equipment appropriate for the animal species, such as solid wall and bottom cages covered with filter bonnets for rodents or other equivalent primary containment systems for larger animal cages.

2. A risk assessment should determine the appropriate type of personal protective equipment to be utilized.

Scrub suits and uniforms are removed before leaving the animal facility. Reusable clothing is appropriately contained and decontaminated before being laundered. Laboratory and protective clothing should never be taken home.

Gowns, uniforms, laboratory coats and personal protective equipment are worn while in the areas where infectious materials and/or animals are housed or manipulated and removed prior to exiting. Disposable personal protective equipment and other contaminated waste are appropriately contained and decontaminated prior to disposal.

3. Eye and face protection (mask, goggles, face shield or other splatter guard) are used for manipulations or activities that may result in splashes or sprays from infectious or other hazardous materials and when the animal or microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates.

Persons having contact with NHPs should assess risk of mucous membrane exposure and wear protective equipment (e.g., masks, goggles, face shields) appropriate for the task to be performed. Respiratory protection is worn based upon risk assessment.

4. Gloves are worn to protect hands from exposure to hazardous materials. A risk assessment should be performed to identify the appropriate glove for the task and alternatives to latex gloves should be available.

Gloves are changed when contaminated, glove integrity is compromised, or when otherwise necessary.

Gloves must not be worn outside the animal rooms.

Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials.

Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste.

Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Hand washing should occur after the removal of gloves.

D. Laboratory Facilities (Secondary Barriers)

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking.

Doors to areas where infectious materials and/or animals are housed, open inward, are self-closing, are kept closed when experimental animals are present, and should never be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.

2. A hand-washing sink is located at the exit of the areas where infectious materials and/or animals are housed or are manipulated. Additional sinks for hand washing should be located in other appropriate locations within the facility.

If the animal facility has segregated areas where infectious materials and/or animals are housed or manipulated, a sink must also be available for hand washing at the exit from each segregated area.

Sink traps are filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.

3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant.

Penetrations in floors, walls and ceiling surfaces are sealed, including openings around ducts, doors and doorframes, to facilitate pest control and proper cleaning.

Floors must be slip-resistant, impervious to liquids, and resistant to chemicals.

4. Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

Furniture should be minimized. Chairs used in animal area must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Sharp edges and corners should be avoided.

5. External windows are not recommended; if present, windows must be sealed and resistant to breakage. The presence of windows may impact facility security and therefore should be assessed by security personnel.
6. Ventilation should be provided in accordance with the *Guide for Care and Use of Laboratory Animals*.¹ The direction of airflow into the animal facility is inward; animal rooms maintain inward directional airflow compared to adjoining hallways. A ducted exhaust air ventilation system is provided. Exhaust air is discharged to the outside without being recirculated to other rooms.

Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.

7. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas, to facilitate cleaning and minimize the accumulation of debris or fomites.
8. Floor drains must be maintained and filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.
9. Cages should be autoclaved or otherwise decontaminated prior to washing. Mechanical cage washer should have a final rinse temperature of at least 180°F. The cage wash area should be designed to accommodate the use of high-pressure spray systems, humidity, strong chemical disinfectants and 180°F water temperatures during the cage/equipment cleaning process.
10. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
11. If BSCs are present, they must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly to the outside through an independent, hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be recertified at least once a year to ensure correct performance.

All BSCs should be used according to manufacturer's specifications to protect the worker and avoid creating a hazardous environment from volatile chemicals and gases.

12. If vacuum service (i.e., central or local) is provided, each service connection should be fitted with liquid disinfectant traps and an in-line HEPA filter placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement.
13. An autoclave should be present in the animal facility to facilitate decontamination of infectious materials and waste.
14. Emergency eyewash and shower are readily available; location is determined by risk assessment.

Animal Biosafety Level 3

Animal Biosafety Level 3 involves practices suitable for work with laboratory animals infected with indigenous or exotic agents, agents that present a potential for aerosol transmission, and agents causing serious or potentially lethal disease. ABSL-3 builds upon the standard practices, procedures, containment equipment, and facility requirements of ABSL-2.

The ABSL-3 laboratory has special engineering and design features.

ABSL-3 requires that: 1) access to the animal facility is restricted; 2) personnel must have specific training in animal facility procedures, the handling of infected animals, and the manipulation of potentially lethal agents; 3) personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations, and husbandry procedures; and 4) procedures involving the manipulation of infectious materials, or where aerosols or splashes may be created, must be conducted in BSCs or by use of other physical containment equipment.

Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment. Employee occupational health programs must be implemented.

The following standard and special safety practices, safety equipment, and facility requirements apply to ABSL-3.

A. Standard Microbiological Practices

1. The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergencies.

Each institute must assure that worker safety and health concerns are addressed as part of the animal protocol review.

Prior to beginning a study, animal protocols must be reviewed and approved by the IACUC⁵ and the Institutional Biosafety Committee.

2. A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety professionals.

The safety manual must be available and accessible. Personnel are advised of potential and special hazards, and are required to read and follow instructions on practices and procedures.

Consideration must be given to specific biohazards unique to the animal species and protocol in use.

3. The supervisor must ensure that animal care, laboratory and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to prevent hazard or exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates or additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.
4. An appropriate medical surveillance program is in place, as determined by risk assessment. The need for an animal allergy prevention program should be considered.

Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with the research, animal husbandry duties, animal care, and manipulations.

Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women of childbearing age should be provided information regarding immune competence and conditions that may predispose them to infection. Individuals having

these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

Personnel using respirators must be enrolled in an appropriately constituted respiratory protection program.

5. A sign incorporating the universal biohazard symbol must be posted at the entrance to areas where infectious materials and/or animals are housed or are manipulated. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of specific infectious agents is recommended when more than one agent is used within an animal room.

Security-sensitive agent information and occupational health requirements should be posted in accordance with the institutional policy.

Advance consideration should be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.^{1,3,4}

6. Access to the animal room is limited to the fewest number of individuals possible. Only those persons required for program or support purposes are authorized to enter the animal facility and the areas where infectious materials and/or animals are housed or are manipulated.

All persons, including facility personnel, service workers, and visitors, are advised of the potential hazards (natural or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.

7. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.

Gloves are worn to prevent skin contact with contaminated, infectious/hazardous materials and when handling animals. Double-glove practices should be used when dictated by risk assessment.

Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated.

Persons must wash their hands after removing gloves and before leaving the areas where infectious materials and/or animals are housed or are manipulated.

Eye, face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.

8. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
9. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
10. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.
11. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented.

When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions must always be taken with sharp items. These include:

- a. Use of needles and syringes or other sharp instruments in the animal facility is limited to situations where there is no alternative such as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
 - b. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used, disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the work site as possible.
 - c. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
 - d. Broken glassware must not be handled directly; it should be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
 - e. Use of equipment with sharp edges and corners should be avoided.
12. Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent, and after any spills, splashes, or other overt contamination.
 13. Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/ or animals are housed or are manipulated.
 14. An effective integrated pest management program is required.
(See Appendix G.)

15. All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof containers for appropriate disposal in compliance with applicable institutional, local and state requirements.

Decontaminate all potentially infectious materials before disposal using an effective method.

B. Special Practices

1. Animal care staff, laboratory and routine support personnel must be provided a medical surveillance program as dictated by the risk assessment and administered appropriate immunizations for agents handled or potentially present, before entry into animal rooms.

When appropriate, a base line serum sample should be stored.

2. All procedures involving the manipulation of infectious materials, handling of infected animals or the generation of aerosols must be conducted within BSCs or other physical containment devices when practical.

When a procedure cannot be performed within a biosafety cabinet, a combination of personal protective equipment and other containment devices must be used.

Restraint devices and practices are used to reduce the risk of exposure during animal manipulations (e.g., physical restraint devices, chemical restraint medications).

3. The risk of infectious aerosols from infected animals or their bedding also can be reduced if animals are housed in containment caging systems, such as solid wall and bottom cages covered with filter bonnets, open cages placed in inward flow ventilated enclosures, HEPA-filter isolators and caging systems, or other equivalent primary containment systems.
4. Actively ventilated caging systems must be designed to prevent the escape of microorganisms from the cage. Exhaust plenums for these systems should be sealed to prevent escape of microorganisms if the ventilation system becomes static, and the exhaust must be HEPA filtered. Safety mechanisms should be in place that prevent the cages and exhaust plenums from becoming positive to the surrounding area should the exhaust fan fail. The system should also be alarmed to indicate operational malfunctions.
5. A method for decontaminating all infectious materials must be available within the facility, preferably within the areas where infectious materials

and/or animals are housed or are manipulated (e.g., autoclave, chemical disinfection, or other approved decontamination methods).

Consideration must be given to means for decontaminating routine husbandry equipment, sensitive electronic and medical equipment.

Decontaminate all potential infectious materials (including animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse) by an appropriate method before removal from the areas where infectious materials and/or animals are housed or manipulated.

It is recommended that animal bedding and waste be decontaminated prior to manipulation and before removal from the areas where infectious materials and/or animals are housed or are manipulated, preferably within the caging system.

Develop and implement an appropriate waste disposal program in compliance with applicable institutional, local and state requirements.

6. Equipment, cages, and racks should be handled in a manner that minimizes contamination of other areas.

Equipment must be decontaminated before repair, maintenance, or removal from the areas where infectious materials and/or animals are housed or are manipulated.

Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.

7. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the safety manual. All such incidents must be reported to the animal facility supervisor or personnel designated by the institution. Medical evaluation, surveillance, and treatment should be provided as appropriate and records maintained.

C. Safety Equipment (*Primary Barriers and Personal Protective Equipment*)

1. Properly maintained BSCs and other physical containment devices or equipment should be used for all manipulations for infectious materials and when possible, animals. These manipulations include necropsy, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals.

The risk of infectious aerosols from infected animals or bedding can be reduced by primary barrier systems. These systems may include solid

wall and bottom cages covered with filter bonnets, ventilated cage rack systems, or for larger cages placed in inward flow ventilated enclosures or other equivalent systems or devices.

2. A risk assessment should determine the appropriate type of personal protective equipment to be utilized.

Personnel within the animal facility where protective clothing, such as uniforms or scrub suits. Reusable clothing is appropriately contained and decontaminated before being laundered. Laboratory and protective clothing should never be taken home. Disposable personal protective equipment such as non-woven olefin cover-all suits, wrap-around or solid-front gowns should be worn over this clothing, before entering the areas where infectious materials and/or animals are housed or manipulated. Front-button laboratory coats are unsuitable.

Disposable personal protective equipment must be removed when leaving the areas where infectious materials and/or animals are housed or are manipulated. Scrub suits and uniforms are removed before leaving the animal facility.

Disposable personal protective equipment and other contaminated waste are appropriately contained and decontaminated prior to disposal.

3. All personnel entering areas where infectious materials and/or animals are housed or manipulated wear appropriate eye, face and respiratory protection. To prevent cross contamination, boots, shoe covers, or other protective footwear, are used where indicated.

Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates.

4. Gloves are worn to protect hands from exposure to hazardous materials.

A risk assessment should be performed to identify the appropriate glove for the task and alternatives to latex gloves should be available.

Procedures may require the use of wearing two pairs of gloves (double-glove).

Gloves are changed when contaminated, glove integrity is compromised, or when otherwise necessary.

Gloves must not be worn outside the animal rooms.

Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials.

Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste.

Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Hand washing should occur after the removal of gloves.

D. Laboratory Facilities (Secondary Barriers)

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking.

Access to the animal facility is restricted.

Doors to areas where infectious materials and/or animals are housed, open inward, are self-closing, are kept closed when experimental animals are present, and should never be propped open.

Entry into the containment area is via a double-door entry, which constitutes an anteroom/airlock and a change room. Showers may be considered based on risk assessment. An additional double-door access anteroom or double-doored autoclave may be provided for movement of supplies and wastes into and out of the facility.

2. A hand-washing sink is located at the exit of the areas where infectious materials and/or animals are housed or are manipulated. Additional sinks for hand washing should be located in other appropriate locations within the facility. The sink should be hands-free or automatically operated.

If the animal facility has multiple segregated areas where infectious materials and/or animals are housed or are manipulated, a sink must also be available for hand washing at the exit from each segregated area.

Sink traps are filled with water, and/or appropriate liquid to prevent the migration of vermin and gases.

3. The animal facility is designed, constructed, and maintained to facilitate cleaning, decontamination and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant.

Penetrations in floors, walls and ceiling surfaces are sealed, including openings around ducts and doorframes, to facilitate pest control, proper cleaning and decontamination. Walls, floors and ceilings should form a sealed and sanitizable surface.

Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Flooring is seamless, sealed resilient or poured floors, with integral cove bases.

Decontamination of an entire animal room should be considered when there has been gross contamination of the space, significant changes in usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the animal room must be based on the risk assessment.

4. Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

Furniture should be minimized. Chairs used in animal areas must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Equipment and furnishings with sharp edges and corners should be avoided.

5. External windows are not recommended; if present, all windows must be sealed and must be resistant to breakage. The presence of windows may impact facility security and therefore should be assessed by security personnel.
6. Ventilation of the facility should be provided in accordance with the *Guide for Care and Use of Laboratory Animals*.¹ The direction of airflow into the animal facility is inward; animal rooms maintain inward directional airflow compared to adjoining hallways. A ducted exhaust air ventilation system is provided. Exhaust air is discharged to the outside without being recirculated to other rooms. This system creates directional airflow, which draws air into the animal room from “clean” areas and toward “contaminated” areas.

Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process. HEPA filtration and other treatments of the exhaust air may not be required, but should be considered based on site requirements, specific agent manipulations and use conditions. The exhaust must be dispersed away from occupied areas and air intakes.

Personnel must verify that the direction of the airflow (into the animal areas) is proper. It is recommended that a visual monitoring device that indicates directional inward airflow be provided at the animal room entry. The ABSL-3 animal facility shall be designed such that under failure

conditions the airflow will not be reversed. Alarms should be considered to notify personnel of ventilation and HVAC system failure.

7. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas, to facilitate cleaning and minimize the accumulation of debris or fomites.
8. Floor drains must be maintained and filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.
9. Cages are washed in a mechanical cage washer. The mechanical cage washer has a final rinse temperature of at least 180°F. Cages should be autoclaved or otherwise decontaminated prior to removal from ABSL-3 space. The cage wash facility should be designed and constructed to accommodate high-pressure spray systems, humidity, strong chemical disinfectants and 180°F water temperatures during the cage cleaning process.
10. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
11. BSCs (Class II, Class III) must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. Class II BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or exhausted directly to the outside through a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be certified at least annually to assure correct performance.

Class III BSCs must supply air in such a manner that prevents positive pressurization of the cabinet or the laboratory room.

All BSCs should be used according to manufacturers' specifications.

When applicable, equipment that may produce infectious aerosols must be contained in devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the animal facility. These HEPA filters should be tested and/or replaced at least annually.

12. An autoclave is available which is convenient to the animal rooms where the biohazard is contained. The autoclave is utilized to decontaminate

infectious materials and waste before moving it to the other areas of the facility. If not convenient to areas where infectious materials and/or animals are housed or are manipulated, special practices should be developed for transport of infectious materials to designated alternate location/s within the facility.

13. Emergency eyewash and shower are readily available; location is determined by risk assessment.
14. The ABSL-3 facility design and operational procedures must be documented. The facility must be tested to verify that the design and operational parameters have been met prior to use. Facilities should be re-verified at least annually against these procedures as modified by operational experience.
15. Additional environmental protection (e.g., personnel showers, HEPA filtration of exhaust air, containment of other piped services, and the provision of effluent decontamination) should be considered if recommended by the agent summary statement, as determined by risk assessment of the site conditions, or other applicable federal, state or local regulations.

Animal Biosafety Level 4

Animal Biosafety Level 4 is required for work with animals infected with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments; or a related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring ABSL-4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level, or to re-designate the level. Animal care staff must have specific and thorough training in handling extremely hazardous, infectious agents and infected animals. Animal care staff must understand the primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics. All animal care staff and supervisors must be competent in handling animals, agents and procedures requiring ABSL-4 containment. The animal facility director and/or laboratory supervisor control access to the animal facility within the ABSL-4 laboratory in accordance with institutional policies.

There are two models for ABSL-4 laboratories:

1. *A Cabinet Laboratory*—All handling of agents, infected animals and housing of infected animals must be performed in Class III BSCs (see Appendix A); and
2. *A Suit Laboratory*—Personnel must wear a positive pressure protective suit (see Appendix A); infected animals must be housed in ventilated enclosures with inward directional airflow and HEPA filtered exhaust;

and infected animals should be handled within a primary barrier system, such as a Class II BSC or other equivalent containment system.

ABSL-4 builds upon the standard practices, procedures, containment equipment, and facility requirements of ABSL-3. However, ABSL-4 cabinet and suit laboratories have special engineering and design features to prevent microorganisms from being disseminated into the environment and personnel. The ABSL-4 cabinet laboratory is distinctly different from an ABSL-3 laboratory containing a Class III BSC. The following standard and special safety practices, equipment, and facilities apply to ABSL-4.

A. *Standard Microbiological Practices*

1. The animal facility directors must establish and enforce policies, procedures, and protocols for biosafety, biosecurity and emergencies within the ABSL-4 laboratory.

The animal facility director and/or designated institutional officials are responsible for enforcing the policies that control access to the ABSL-4 facility. Laboratory personnel and support staff must be provided appropriate occupational medical service including medical surveillance and available immunizations for agents handled or potentially present in the laboratory.³ A system must be established for reporting and documenting laboratory accidents, exposures, employee absenteeism and for the medical surveillance of potential laboratory-associated illnesses. An essential adjunct to such an occupational medical services system is the availability of a facility for the isolation and medical care of personnel with potential or known laboratory-acquired infections. Facility supervisors should ensure that medical staff are informed of potential occupational hazards within the animal facility including those associated with the research, animal husbandry duties, animal care, and manipulations.

An ABSL-4 laboratory specific, biosafety manual must be prepared in consultation with the animal facility director, the laboratory supervisor, and the biosafety advisor. The biosafety manual must be available and accessible. Personnel are advised of special hazards, and are required to read and follow instructions on practices and procedures.

Prior to beginning a study, appropriate policies and procedures for animal welfare during the conduct of research, must be developed and approved by the IACUC. The biosafety official, the IBC and/or other applicable committees, are responsible for review of protocols and policies to prevent hazardous exposures to personnel who manipulate and care for animals.

2. A complete clothing change is required in the ABSL-4 operation. Personnel within the animal facility where protective clothing, such as uniforms or scrub suits.

All persons leaving the BSL-4/ABSL-4 laboratory are required to take a personal body shower.

3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented.

When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:

- a. Use of needles and syringes or other sharp instruments are limited for use in the animal facility is limited to situations where there is no alternative such as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
 - b. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal and placed as close to the work site as possible.
 - c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
 - d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
 - e. Equipment containing sharp edges and corners should be avoided.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.

Procedures involving the manipulation of infectious materials must be conducted within biological safety cabinets, or other physical containment devices. When procedures cannot be performed in a BSC, alternate containment equipment should be used.

7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.

Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All incidents must be reported to the animal facility director, laboratory supervisor, institutional management and appropriate facility safety personnel. Medical evaluation, surveillance, and treatment must be provided and appropriate records maintained.

8. Decontaminate all wastes (including animal tissues, carcasses, and contaminated bedding) and other materials before removal from the ABSL-4 laboratory by an effective and validated method. Laboratory clothing should be decontaminated before laundering.

Supplies and materials needed in the facility must be brought in through a double-door autoclave, fumigation chamber, or airlock. Supplies and materials that are not brought into the ABSL-4 laboratory through the change room must be brought in through a previously decontaminated double-door autoclave, fumigation chamber, or airlock. Containment should be maintained at all times. After securing the outer doors, personnel within the areas where infectious materials and/or animals are housed or are manipulated retrieve the materials by opening the interior doors of the autoclave, fumigation chamber, or airlock. These doors must be secured after materials are brought into the facility.

Only necessary equipment and supplies should be taken inside the ABSL-4 laboratory. All equipment and supplies taken inside the laboratory must be decontaminated before removal. Consideration should be given to means for decontaminating routine husbandry equipment and sensitive electronic and medical equipment.

The doors of the autoclave and fumigation chamber are interlocked in a manner that prevents opening of the outer door unless the autoclave has been operated through a decontamination cycle or the fumigation chamber has been decontaminated.

9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory and the animal room/s when infectious agents are present. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of specific infectious agents is recommended when more than one agent is being used within an animal room.

Security sensitive agent information and occupational health requirements should be posted in accordance with the institutional policy.

Advance consideration must be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.^{1,3,4}

10. An effective integrated pest management program is required.
(See Appendix G.)
11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.
12. Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/ or animals are housed or are manipulated.

B. Special Practices

1. All persons entering the ABSL-4 laboratory must be advised of the potential hazards and meet specific entry/exit requirements.

Only persons whose presence in the laboratory or individual animal rooms is required for scientific or support purposes are authorized to enter.

Entry into the facility must be limited by means of secure, locked doors. A logbook, or other means of documenting the date and time of all persons entering and leaving the ABSL-4 laboratory must be maintained.

While the laboratory is operational, personnel must enter and exit the laboratory through the clothing change and shower rooms except during emergencies. All personal clothing must be removed in the outer clothing change room. All personnel entering the laboratory must use laboratory clothing, including undergarments, pants, shirts, jumpsuits, shoes, and gloves.

All persons leaving the ABSL-4 laboratory are required to take a personal body shower. Used laboratory clothing must not be removed

from the inner change room through the personal shower. These items must be treated as contaminated materials and decontaminated before laundering or disposal.

After the laboratory has been completely decontaminated by validated method, necessary staff may enter and exit the laboratory without following the clothing change and shower requirements described above.

Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

2. Animal facility personnel and support staff must be provided occupational medical services, including medical surveillance and available immunizations for agents handled or potentially present in the laboratory. A system must be established for reporting and documenting laboratory accidents, exposures, employee absenteeism and for the medical surveillance of potential laboratory-acquired illnesses. An essential adjunct to an occupational medical system is the availability of a facility for the isolation and medical care of personnel with potential or known laboratory-acquired illnesses.
3. Each institution must establish policies and procedures describing the collection and storage of serum samples from at-risk personnel.
4. The animal facility supervisor is responsible for ensuring that animal personnel:
 - a. Receive appropriate training in the practices and operations specific to the animal facility, such as animal husbandry procedures, potential hazards present, manipulations of infectious agents, and necessary precautions to prevent potential exposures.
 - b. Demonstrate high proficiency in standard and special microbiological practices, and techniques before entering the ABSL-4 facility or working with agents requiring ABSL-4 containment.
 - c. Receive annual updates and additional training when procedure or policy changes occur. Records are maintained for all hazard evaluations and employee training.
5. Removal of biological materials that are to remain in a viable or intact state from the ABSL-4 laboratory must be transferred to a non-breakable,

sealed primary container and then enclosed in a non-breakable, sealed secondary container. These materials must be transferred through a disinfectant dunk tank, fumigation chamber, or decontamination shower. Once removed, packaged viable material must not be opened outside ABSL-4 containment unless inactivated by a validated method.

6. Laboratory equipment must be routinely decontaminated, as well as after spills, splashes, or other potential contamination. Equipment, cages, and racks should be handled in manner that minimizes contamination of other areas. Cages are autoclaved or thoroughly decontaminated before they are cleaned and washed.
 - a. All equipment and contaminated materials must be decontaminated before removal from the animal facility. Equipment must be decontaminated using an effective and validated method before repair, maintenance, or removal from the animal facility.
 - b. Equipment or material that might be damaged by high temperatures or steam must be decontaminated using an effective and validated procedure such as a gaseous or vapor method in an airlock or chamber designed for this purpose.
 - c. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material. A spill procedure must be developed and posted within the laboratory. Spills and accidents of potentially infectious materials must be immediately reported to the animal facility and laboratory supervisors or personnel designated by the institution.
7. The doors of the autoclave and fumigation chamber are interlocked in a manner that prevents opening of the outer door unless the autoclave/ decontamination chamber has been operated through a decontamination cycle or the fumigation chamber has been decontaminated.
8. Daily inspections of essential containment and life support systems must be completed before laboratory work is initiated to ensure that the laboratory and animal facilities are operating according to established parameters.
9. Practical and effective protocols for emergencies must be established. These protocols must include plans for medical emergencies, facility malfunctions, fires, escape of animals within the ABSL-4 laboratory, and other potential emergencies. Training in emergency response procedures must be provided to emergency response personnel according to institutional policies.

10. Based on site-specific risk assessment, personnel assigned to work with infected animals may be required to work in pairs. Procedures to reduce possible worker exposure must be instituted, such as use of squeeze cages, working only with anesthetized animals, or other appropriate practices.

C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

Cabinet Laboratory

1. All manipulations of infectious animals and materials within the laboratory must be conducted in the Class III BSC. Double-door, pass through autoclaves must be provided for decontaminating materials passing out of the Class III BSC(s). The autoclave doors must be interlocked so that only one can be opened at any time and be automatically controlled so that the outside door to the autoclave can only be opened after the decontamination cycle has been completed.

The Class III cabinet must also have a pass-through dunk tank, fumigation chamber, or equivalent decontamination method so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the cabinet. Containment must be maintained at all times.

The Class III cabinet must have a HEPA filter on the supply air intake and two HEPA filters in series on the exhaust outlet of the unit. There must be gas-tight dampers on the supply and exhaust ducts of the cabinet to permit gas or vapor decontamination of the unit. Ports for injection of test medium must be present on all HEPA filter housings.

The interior of the Class III cabinet must be constructed with smooth finishes that can be easily cleaned and decontaminated. All sharp edges on cabinet finishes must be eliminated to reduce the potential for cuts and tears of gloves. Equipment to be placed in the Class III cabinet should also be free of sharp edges or other surfaces that may damage or puncture the cabinet gloves.

Class III cabinet gloves must be inspected for leaks periodically and changed if necessary. Gloves should be replaced annually during cabinet re-certification.

The cabinet should be designed to permit maintenance and repairs of cabinet mechanical systems (refrigeration, incubators, centrifuges, etc.) to be performed from the exterior of the cabinet whenever possible.

Manipulation of high concentrations or large volumes of infectious agents within the Class III cabinet should be performed using physical containment devices inside the cabinet whenever practical. Such

materials should be centrifuged inside the cabinet using sealed rotor heads or centrifuge safety cups.

The interior of the Class III cabinet as well as all contaminated plenums, fans and filters must be decontaminated using a validated gaseous or vapor method.

The Class III cabinet must be certified at least annually.

Restraint devices and practices that reduce the risk of exposure during animal manipulations must be used where practicable (e.g., physical restraint devices, chemical restraint medications, mesh or Kevlar gloves, etc.).

2. Workers must wear protective laboratory clothing such as solid-front or wrap-around gowns, scrub suits, or coveralls when in the laboratory. No personal clothing, jewelry, or other items except eyeglasses should be taken past the personal shower area. Upon exiting the laboratory, all protective clothing must be removed in the dirty side change room before showering. Reusable laboratory clothing must be autoclaved before being laundered.
3. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment. Prescription eye glasses must be decontaminated before removal through the personal body shower.
4. Gloves must be worn to protect against breaks or tears in the cabinet gloves. Gloves must not be worn outside the laboratory. Alternatives to latex gloves should be available. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste.

Suit Laboratory

1. Infected animals should be housed in a primary containment system (such as open cages placed in ventilated enclosures, solid wall and bottom cages covered with filter bonnets and opened in laminar flow hoods, or other equivalent primary containment systems).

Personnel wearing a one-piece positive pressure suit ventilated with a life support system must conduct all procedures.

All manipulations of potentially infectious agents must be performed within a Class II BSC or other primary barrier system. Infected animals should be handled within a primary barrier system, such as a Class II BSC or other equivalent containment system.

Equipment that may produce aerosols must be contained in devices that exhaust air through HEPA filtration before being discharged into the laboratory. These HEPA filters should be tested annually and replaced as need.

HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations.

2. Workers must wear protective laboratory clothing, such as scrub suits, before entering the room used for donning positive pressure suits. All protective clothing must be removed in the dirty side change room before entering the personal shower. Reusable laboratory clothing must be autoclaved before being laundered.
3. Inner gloves must be worn to protect against break or tears in the outer suit gloves. Disposable gloves must not be worn outside the change area. Alternatives to latex gloves should be available. Do not wash or reuse disposable gloves. Inner gloves must be removed and discarded in the inner change room prior to entering the personal shower. Dispose of used gloves with other contaminated waste.
4. Decontamination of outer suit gloves is performed during operations to remove gross contamination and minimize further contamination of the laboratory.

D. Laboratory Facilities (Secondary Barriers)

Cabinet Laboratory

1. The ABSL-4 cabinet laboratory consists of either a separate building or a clearly demarcated and isolated zone within a building. Laboratory doors must have locks in accordance with the institutional policies.

Rooms in the ABSL-4 facility must be arranged to ensure sequential passage through an inner (dirty) change area, personal shower and outer (clean) change room prior to exiting the room(s) containing the Class III BSC(s).

An automatically activated emergency power source must be provided at a minimum for the laboratory exhaust system, life support systems, alarms, lighting, entry and exit controls, BSCs, and door gaskets. Monitoring and control systems for air supply, exhaust, life support, alarms, entry and exit, and security systems should be on an uninterrupted power supply (UPS).

A double-door autoclave, dunk tank, fumigation chamber, or ventilated anteroom/airlock must be provided at the containment barrier for the passage of materials, supplies, or equipment.

2. A hands-free sink must be provided near the doors of the cabinet room(s) and the inner change rooms. A sink must be provided in the outer change room. All sinks in the room(s) containing the Class III BSC must be connected to the wastewater decontamination system.
3. Walls, floors, and ceilings of the laboratory must be constructed to form a sealed internal shell to facilitate fumigation and prohibit animal and insect intrusion. The internal surfaces of this shell must be resistant to liquids and chemicals used for cleaning and decontamination of the area. Floors must be monolithic, sealed and coved.

All penetrations in the internal shell of the laboratory and inner change room must be sealed.

Openings around doors into the cabinet room and inner change room must be minimized and capable of being sealed to facilitate decontamination.

All drains in ABSL-4 laboratory area floor must be connected directly to the liquid waste decontamination system.

Services and plumbing that penetrate the laboratory walls, floors or ceiling, must be installed to ensure that no backflow from the laboratory occurs. Services must be sealed and be provided with redundant backflow prevention. Consideration should be given to locating these devices outside of containment. Atmospheric venting systems must be provided with two HEPA filters in series and are sealed up to the second filter.

Decontamination of the entire cabinet must be performed using a validated gaseous or vapor method when there have been significant changes in cabinet usage, before major renovations or maintenance shut downs, and in other situations, as determined by risk assessment. Selection of the appropriate materials and methods used for decontamination must be based on the risk assessment of the biological agents in use.

4. Laboratory furniture must be of simple construction, capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning and decontamination. Chairs and other furniture should be covered with a non-porous material that can be easily decontaminated.

5. Windows must be break-resistant and sealed.
6. If Class II BSCs are needed in the cabinet laboratory, they must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. Class II BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Central vacuum systems are not recommended. If, however, there is a central vacuum system, it must not serve areas outside the cabinet room. Two in-line HEPA filters must be placed near each use point. Filters must be installed to permit in-place decontamination and replacement.
8. An eyewash station must be readily available in the laboratory.
9. A dedicated non-recirculating ventilation system is provided. Only laboratories with the same HVAC requirements (i.e., other BSL-4 labs, ABSL-4, BSL-3-Ag labs) may share ventilation systems if gas-tight dampers and HEPA filters isolate each individual laboratory system.

The supply and exhaust components of the ventilation system must be designed to maintain the ABSL-4 laboratory at negative pressure to surrounding areas and provide differential pressure/directional airflow between adjacent areas within the laboratory.

Redundant supply fans are recommended. Redundant exhaust fans are required. Supply and exhaust fans must be interlocked to prevent positive pressurization of the laboratory.

The ventilation system must be monitored and alarmed to indicate malfunction or deviation from design parameters. A visual monitoring device must be installed near the clean change room so proper differential pressures within the laboratory may be verified.

Supply air to and exhaust air from the cabinet room and fumigation/decontamination chambers must pass through HEPA filter(s). The air exhaust discharge must be located away from occupied spaces and building air intakes.

All HEPA filters should be located as near as practicable to the cabinet laboratory in order to minimize the length of potentially contaminated ductwork. All HEPA filters must be tested and certified annually.

The HEPA filter housings should be designed to allow for *in situ* decontamination and validation of the filter prior to removal. The design of the HEPA filter housing must have gas-tight isolation dampers; decontamination ports, and ability to scan each filter assembly for leaks.

10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to the manufacturer's recommendations. Biological safety cabinets can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.

Class III BSCs must be directly and independently exhausted through two HEPA filters in series. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.

11. Pass through dunk tanks, fumigation chambers, or equivalent decontamination methods must be provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the cabinet room(s). Access to the exit side of the pass through shall be limited to those individuals authorized to be in the ABSL-4 laboratory.
12. Liquid effluents from cabinet room sinks, floor drains, autoclave chambers, and other sources within the cabinet room must be decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer.

Decontamination of all liquid wastes must be documented. The decontamination process for liquid wastes must be validated physically and biologically. Biological validation must be performed annually or more often as required by institutional policy.

Effluents from showers and toilets may be discharged to the sanitary sewer without treatment.

13. A double-door autoclave must be provided for decontaminating waste or other materials passing out of the cabinet room. Autoclaves that open outside of the laboratory must be sealed to the wall. This bioseal must be durable, airtight, and sealed to the wall. Positioning the bioseal so that the equipment can be accessed and maintained from outside the laboratory is recommended. The autoclave doors must be interlocked so that only one can be opened at any time and be automatically controlled so that the outside door can only be opened after the autoclave decontamination cycle has been completed.

Gas and liquid discharge from the autoclave chamber must be decontaminated. When feasible, autoclave decontamination processes should be designed so that over-pressurization cannot release unfiltered air or steam exposed to infectious material to the environment.

14. The ABSL-4 facility design parameters and operational procedures must be documented. The facility must be tested to verify that the design and operational parameters have been met prior to operation. Facilities must also be re-verified annually. Verification criteria should be modified as necessary by operational experience.
15. Appropriate communication systems must be provided between the ABSL-4 laboratory and the outside (e.g., voice, fax, and computer). Provisions for emergency communication and access/egress must be considered.

Suit Laboratory

1. The ABSL-4 suit laboratory consists of either a separate building or a clearly demarcated and isolated zone within a building. Laboratory doors must have locks in accordance with the institutional policies.

Entry to this laboratory must be through an airlock fitted with airtight doors. Personnel who enter this laboratory must wear a positive pressure suit ventilated by a life support system with HEPA filtered breathing air. The breathing air system must have redundant compressors, failure alarms and an emergency backup system.

Rooms in the facility must be arranged to ensure sequential passage through the chemical shower, inner (dirty) change room, personal shower, and outer (clean) changing area upon exit.

A chemical shower must be provided to decontaminate the surface of the positive pressure suit before the worker leaves the ABSL-4 laboratory. In the event of an emergency exit or failure of chemical shower, a method for decontaminating positive pressure suits, such as a gravity fed supply of chemical disinfectant, is needed.

An automatically activated emergency power source must be provided at a minimum for the laboratory exhaust system, life support systems, alarms, lighting, entry and exit controls, BSCs, and door gaskets. Monitoring and control systems for air supply, exhaust, life support, alarms, entry and exit, and security systems should be on a UPS.

A double-door autoclave, dunk tank, or fumigation chamber must be provided at the containment barrier for the passage of materials, supplies, or equipment.

2. Sinks inside the ABSL-4 laboratory must be placed near procedure areas, contain traps, and be connected to the wastewater decontamination system.

3. Walls, floors, and ceilings of the ABSL-4 laboratory must be constructed to form a sealed internal shell to facilitate fumigation and prohibit animal and insect intrusion. The internal surfaces of this shell must be resistant to liquids and chemicals used for cleaning and decontamination of the area. Floors must be monolithic, sealed and coved.

All penetrations in the internal shell of the laboratory, suit storage room and the inner change room must be sealed.

Drains, if present, in the laboratory floor must be connected directly to the liquid waste decontamination system. Sewer vents and other service lines must be protected by two HEPA filters in series and have protection against insect and animal intrusion.

Services and plumbing that penetrate the laboratory walls, floors, or ceiling must be installed to ensure that no backflow from the laboratory occurs. These penetrations must be fitted with two (in series) backflow prevention devices. Consideration should be given to locating these devices outside of containment. Atmospheric venting systems must be provided with two HEPA filters in series and be sealed up to the second filter.

Decontamination of the entire laboratory must be performed using a validated gaseous or vapor method when there have been significant changes in laboratory usage, before major renovations or maintenance shut downs, and in other situations, as determined by risk assessment.

4. Laboratory furniture must be of simple construction, capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning, decontamination and unencumbered movement of personnel. Chairs and other furniture should be covered with a non-porous material that can be easily decontaminated. Sharp edges and corners should be avoided.
5. Windows must be break-resistant and sealed.
6. BSCs and other primary containment barrier systems must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Central vacuum systems are not recommended. If, however, there is a central vacuum system, it must not serve areas outside the ABSL-4 laboratory. Two in-line HEPA filters must be placed near each use point. Filters must be installed to permit in-place decontamination and replacement.

8. An eyewash station must be readily available in the laboratory area for use during maintenance and repair activities.
9. A dedicated non-recirculating ventilation system is provided. Only laboratories with the same HVAC requirements (i.e., other BSL-4 labs, ABSL-4, BSL-3-Ag labs) may share ventilation systems if gas tight dampers and HEPA filters isolate each individual laboratory system.

The supply and exhaust components of the ventilation system must be designed to maintain the BSL-4/ABSL-4 laboratory at negative pressure to surrounding areas and provide correct differential pressure between adjacent areas within the laboratory.

Redundant supply fans are recommended. Redundant exhaust fans are required. Supply and exhaust fans must be interlocked to prevent positive pressurization of the laboratory.

The ventilation system must be monitored and alarmed to indicate malfunction or deviation from design parameters. A visual monitoring device must be installed near the clean change room so proper differential pressures within the laboratory may be verified.

Supply air to the ABSL-4 laboratory, including the decontamination shower, must pass through a HEPA filter. All exhaust air from the BSL-4/ABSL-4 suit laboratory, decontamination shower and fumigation or decontamination chambers must pass through two HEPA filters, in series before discharge to the outside. The exhaust air discharge must be located away from occupied spaces and air intakes.

All HEPA filters must be located as near as practicable to the areas where infectious materials and/or animals are housed or are manipulated in order to minimize the length of potentially contaminated ductwork. All HEPA filters must be tested and certified annually.

The HEPA filter housings are designed to allow for *in situ* decontamination and validation of the filter prior to removal. The design of the HEPA filter housing must have gas-tight isolation dampers; decontamination ports; and ability to scan each filter assembly for leaks.

10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to the manufacturer's recommendations. Biological safety cabinets can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly to the outside through an independent, direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.

11. Pass through dunk tanks, fumigation chambers, or equivalent decontamination methods must be provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the ABSL-4 laboratory. Access to the exit side of the pass-through shall be limited to those individuals authorized to be in the ABSL-4 laboratory.
12. Liquid effluents from chemical showers, sinks, floor drains, autoclave chambers, and other sources within the laboratory must be decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer.

Decontamination of all liquid wastes must be documented. The decontamination process for liquid wastes must be validated physically and biologically. Biological validation must be performed annually or more often as required by institutional policy.

Effluents from personal body showers and toilets may be discharged to the sanitary sewer without treatment.

13. A double-door, pass through autoclave(s) must be provided for decontaminating materials passing out of the cabinet laboratory. Autoclaves that open outside of the laboratory must be sealed to the wall through which the autoclave passes. This bioseal must be durable and airtight. Positioning the bioseal so that the equipment can be accessed and maintained from outside the laboratory is strongly recommended. The autoclave doors must be interlocked so that only one can be opened at any time and be automatically controlled so that the outside door to the autoclave can only be opened after the decontamination cycle has been completed.

The size of the autoclave should be sufficient to accommodate the intended usage, equipment size, and potential future increases in cage size. Autoclaves should facilitate isolation for routine servicing.

Gas and liquid discharge from the autoclave chamber must be decontaminated. When feasible, autoclave decontamination processes should be designed so that over-pressurization cannot release unfiltered air or steam exposed to infectious material to the environment.

14. The ABSL-4 facility design parameters and operational procedures must be documented. The facility must be tested to verify that the design and operational parameters have been met prior to operation. Facilities must also be re-verified. Verification criteria should be modified as necessary by operational experience.

Consider placing ABSL-4 areas away from exterior walls of buildings to minimize the impact from the outside environmental and temperatures.

15. Appropriate communication systems must be provided between the laboratory and the outside (e.g., voice, fax, and computer). Provisions for emergency communication and access/egress should be considered.

References

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Table 3. Summary of Recommended Animal Biosafety Levels for Activities in which Experimentally or Naturally Infected Vertebrate Animals are Used

BSL	Agents	Practices	Primary Barriers and Safety Equipment	Facilities (Secondary Barriers)
1	Not known to consistently cause diseases in healthy adults	Standard animal care and management practices, including appropriate medical surveillance programs	As required for normal care of each species <ul style="list-style-type: none"> ■ PPE: laboratory coats and gloves; eye, face protection, as needed 	Standard animal facility: <ul style="list-style-type: none"> ■ No recirculation of exhaust air ■ Directional air flow recommended ■ Hand washing sink is available
2	<ul style="list-style-type: none"> ■ Agents associated with human disease ■ Hazard: percutaneous injury, ingestion, mucous membrane exposure 	ABSL-1 practice plus: <ul style="list-style-type: none"> ■ Limited access ■ Biohazard warning signs ■ "Sharps" precautions ■ Biosafety manual ■ Decontamination of all infectious wastes and animal cages prior to washing 	ABSL-1 equipment plus primary barriers: <ul style="list-style-type: none"> ■ Containment equipment appropriate for animal special ■ PPE: Laboratory coats, gloves, face, eye and respiratory protection, as needed ■ 	ABSL-1 plus: <ul style="list-style-type: none"> ■ Autoclave available ■ Hand washing sink available ■ Mechanical cage washer recommended ■ Negative airflow into animal and procedure rooms recommended
3	Indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure	ABSL-2 practice plus: <ul style="list-style-type: none"> ■ Controlled access ■ Decontamination of clothing before laundering ■ Cages decontaminated before bedding is removed ■ Disinfectant foot bath as needed 	ABSL-2 equipment plus: <ul style="list-style-type: none"> ■ Containment equipment for housing animals and cage dumping activities ■ Class I, II or III BSCs available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols ■ PPE: Appropriate respiratory protection 	ABSL-2 facility plus: <ul style="list-style-type: none"> ■ Physical separation from access corridors ■ Self-closing, double-door access ■ Sealed penetrations ■ Sealed windows ■ Autoclave available in facility ■ Entry through ante-room or airlock ■ Negative airflow into animal and procedure rooms ■ Hand washing sink near exit of animal or procedure room
4	<ul style="list-style-type: none"> ■ Dangerous/exotic agents which post high risk of aerosol transmitted laboratory infections that are frequently fatal, for which there are no vaccines or treatments ■ Agents with a close or identical antigenic relationship to an agent requiring BSL-4 until data are available to redesignate the level ■ Related agents with unknown risk of transmission 	ABSL-3 practices plus: <ul style="list-style-type: none"> ■ Entrance through change room where personal clothing is removed and laboratory clothing is put on; shower on exiting ■ All wastes are decontaminated before removal from the facility 	ABSL-3 equipment plus: <ul style="list-style-type: none"> ■ Maximum containment equipment (i.e., Class III BSC or partial containment equipment in combination with full body, air-supplied positive-pressure suit) used for all procedures and activities 	ABSL-3 facility plus: <ul style="list-style-type: none"> ■ Separate building or isolated zone ■ Dedicated supply and exhaust, vacuum, and decontamination systems ■ Other requirements outlined in the text

Section VI—Principles of Laboratory Biosecurity

Since the publication of the 4th edition of BMBL in 1999, significant events have brought national and international scrutiny to the area of laboratory security. These events, including the anthrax attacks on U.S. citizens in October 2001 and the subsequent expansion of the United States Select Agent regulations in December 2003, have led scientists, laboratory managers, security specialists, biosafety professionals, and other scientific and institutional leaders to consider the need for developing, implementing and/or improving the security of biological agents and toxins within their facilities. Appendix F of BMBL 4th edition provided a brief outline of issues to consider in developing a security plan for biological agents and toxins capable of serious or fatal illness to humans or animals. In December 2002, Appendix F was updated and revised as a security and emergency response guidance for laboratories working with select agents.¹ Section VI replaces the previous appendices. The current Appendix F discusses Select Agent and Toxin regulations.

This section describes laboratory biosecurity planning for microbiological laboratories. As indicated below, laboratories with good biosafety programs already fulfill many of the basic requirements for security of biological materials. For laboratories not handling select agents, the access controls and training requirements specified for BSL-2 and BSL-3 in BMBL may provide sufficient security for the materials being studied. Security assessments and additional security measures should be considered when select agents, other agents of high public health and agriculture concern, or agents of high commercial value such as patented vaccine candidates, are introduced into the laboratory.

The recommendations presented in this section are advisory. Excluding the Select Agent regulations, there is no current federal requirement for the development of a biosecurity program. However, the application of these principles and the assessment process may enhance overall laboratory management. Laboratories that fall under the Select Agent regulations should consult Appendix F (42 CFR part 73; 7 CFR 331 and 9 CFR 121).^{4,5,6}

The term “biosecurity” has multiple definitions. In the animal industry, the term biosecurity relates to the protection of an animal colony from microbial contamination. In some countries, the term biosecurity is used in place of the term biosafety. For the purposes of this section the term “biosecurity” will refer to the protection of microbial agents from loss, theft, diversion or intentional misuse. This is consistent with current WHO and American Biological Safety Association (ABSA) usage of this term.^{2,3}

Security is not a new concept in biological research and medical laboratories. Several of the security measures discussed in this section are embedded in the biosafety levels that serve as the foundation for good laboratory practices throughout the biological laboratory community. Most biomedical and

microbiological laboratories do not have select agents or toxins, yet maintain control over and account for research materials, protect relevant sensitive information, and work in facilities with access controls commensurate with the potential public health and economic impact of the biological agents in their collections. These measures are in place in most laboratories that apply good laboratory management practices and have appropriate biosafety programs.

Biosafety and Biosecurity

Biosafety and biosecurity are related, but not identical, concepts. Biosafety programs reduce or eliminate exposure of individuals and the environment to potentially hazardous biological agents. Biosafety is achieved by implementing various degrees of laboratory control and containment, through laboratory design and access restrictions, personnel expertise and training, use of containment equipment, and safe methods of managing infectious materials in a laboratory setting.

The objective of biosecurity is to prevent loss, theft or misuse of microorganisms, biological materials, and research-related information. This is accomplished by limiting access to facilities, research materials and information. While the objectives are different, biosafety and biosecurity measures are usually complementary.

Biosafety and biosecurity programs share common components. Both are based upon risk assessment and management methodology; personnel expertise and responsibility; control and accountability for research materials including microorganisms and culture stocks; access control elements; material transfer documentation; training; emergency planning; and program management.

Biosafety and biosecurity program risk assessments are performed to determine the appropriate levels of controls within each program. Biosafety looks at appropriate laboratory procedures and practices necessary to prevent exposures and occupationally-acquired infections, while biosecurity addresses procedures and practices to ensure that biological materials and relevant sensitive information remain secure.

Both programs assess personnel qualifications. The biosafety program ensures that staff are qualified to perform their jobs safely through training and documentation of technical expertise. Staff must exhibit the appropriate level of professional responsibility for management of research materials by adherence to appropriate materials management procedures. Biosafety practices require laboratory access to be limited when work is in progress. Biosecurity practices ensure that access to the laboratory facility and biological materials are limited and controlled as necessary. An inventory or material management process for control and tracking of biological stocks or other sensitive materials is also a component of both programs. For biosafety, the shipment of infectious biological materials must adhere to safe packaging, containment and appropriate transport procedures, while biosecurity ensures that transfers are controlled, tracked and

documented commensurate with the potential risks. Both programs must engage laboratory personnel in the development of practices and procedures that fulfill the biosafety and biosecurity program objectives but that do not hinder research or clinical/diagnostic activities. The success of both of these programs hinges on a laboratory culture that understands and accepts the rationale for biosafety and biosecurity programs and the corresponding management oversight.

In some cases, biosecurity practices may conflict with biosafety practices, requiring personnel and management to devise policies that accommodate both sets of objectives. For example, signage may present a conflict between the two programs. Standard biosafety practice requires that signage be posted on laboratory doors to alert people to the hazards that may be present within the laboratory. The biohazard sign normally includes the name of the agent, specific hazards associated with the use or handling of the agent and contact information for the investigator. These practices may conflict with security objectives. Therefore, biosafety and biosecurity considerations must be balanced and proportional to the identified risks when developing institutional policies.

Designing a biosecurity program that does not jeopardize laboratory operations or interfere with the conduct of research requires a familiarity with microbiology and the materials that require protection. Protecting pathogens and other sensitive biological materials while preserving the free exchange of research materials and information may present significant institutional challenges. Therefore, a combination or tiered approach to protecting biological materials, commensurate with the identified risks, often provides the best resolution to conflicts that may arise. However, in the absence of legal requirements for a biosecurity program, the health and safety of laboratory personnel and the surrounding environment should take precedence over biosecurity concerns.

Risk Management Methodology

A risk management methodology can be used to identify the need for a biosecurity program. A risk management approach to laboratory biosecurity 1) establishes which, if any, agents require biosecurity measures to prevent loss, theft, diversion, or intentional misuse, and 2) ensures that the protective measures provided, and the costs associated with that protection, are proportional to the risk. The need for a biosecurity program should be based on the possible impact of the theft, loss, diversion, or intentional misuse of the materials, recognizing that different agents and toxins will pose different levels of risk. Resources are not infinite. Biosecurity policies and procedures should not seek to protect against every conceivable risk. The risks need to be identified, prioritized and resources allocated based on that prioritization. Not all institutions will rank the same agent at the same risk level. Risk management methodology takes into consideration available institutional resources and the risk tolerance of the institution.

Developing a Biosecurity Program

Management, researchers and laboratory supervisors must be committed to being responsible stewards of infectious agents and toxins. Development of a biosecurity program should be a collaborative process involving all stakeholders. The stakeholders include but are not limited to: senior management; scientific staff; human resource officials; information technology staff; and safety, security and engineering officials. The involvement of organizations and/or personnel responsible for a facility's overall security is critical because many potential biosecurity measures may already be in place as part of an existing safety or security program. This coordinated approach is critical in ensuring that the biosecurity program provides reasonable, timely and cost-effective solutions addressing the identified security risks without unduly affecting the scientific or business enterprise or provision of clinical and/or diagnostic services.

The need for a biosecurity program should reflect sound risk management practices based on a site-specific risk assessment. A biosecurity risk assessment should analyze the probability and consequences of loss, theft and potential misuse of pathogens and toxins.⁷ Most importantly, the biosecurity risk assessment should be used as the basis for making risk management decisions.

Example Guidance: A Biosecurity Risk Assessment and Management Process

Different models exist regarding biosecurity risk assessment. Most models share common components such as asset identification, threat, vulnerability and mitigation. What follows is one example of how a biosecurity risk assessment may be conducted. In this example, the entire risk assessment and risk management process may be divided into five main steps, each of which can be further subdivided: 1) identify and prioritize biologicals and/or toxins; 2) identify and prioritize the adversary/threat to biologicals and/or toxins; 3) analyze the risk of specific security scenarios; 4) design and develop an overall risk management program; and 5) regularly evaluate the institution's risk posture and protection objectives. Example guidance for these five steps is provided below.

Step 1: Identify and Prioritize Biological Materials

- Identify the biological materials that exist at the institution, form of the material, location and quantities, including non-replicating materials (i.e., toxins).
- Evaluate the potential for misuse of these biologic materials.
- Evaluate the consequences of misuse of these biologic materials.
- Prioritize the biologic materials based on the consequences of misuse (i.e., risk of malicious use).

At this point, an institution may find that none of its biologic materials merit the development and implementation of a separate biosecurity program or the existing security at the facility is adequate. In this event, no additional steps would need to be completed.

Step 2: Identify and Prioritize the Threat to Biological Materials

- Identify the types of “Insiders” who may pose a threat to the biologic materials at the institution.
- Identify the types of “Outsiders” (if any) who may pose a threat to the biologic materials at the institution.
- Evaluate the motive, means, and opportunity of these various potential adversaries.

Step 3: Analyze the Risk of Specific Security Scenarios

- Develop a list of possible biosecurity scenarios, or undesired events that could occur at the institution (each scenario is a combination of an agent, an adversary, and an action). Consider:
 - access to the agent within your laboratory;
 - how the undesired event could occur;
 - protective measures in place to prevent occurrence;
 - how the existing protection measures could be breached (i.e., vulnerabilities).
- Evaluate the probability of each scenario materializing (i.e., the likelihood) and its associated consequences. Assumptions include:
 - although a wide range of threats are possible, certain threats are more probable than others;
 - all agents/assets are not equally attractive to an adversary; valid and credible threats, existing precautions, and the potential need for select enhanced precautions are considered.
- Prioritize or rank the scenarios by risk for review by management.

Step 4: Develop an Overall Risk Management Program

- Management commits to oversight, implementation, training and maintenance of the biosecurity program.
- Management develops a biosecurity risk statement, documenting which biosecurity scenarios represent an unacceptable risk and must be mitigated versus those risks appropriately handled through existing protection controls.

- Management develops a biosecurity plan to describe how the institution will mitigate those unacceptable risks including:
 - a written security plan, standard operating procedures, and incident response plans;
 - written protocols for employee training on potential hazards, the biosecurity program and incident response plans.
- Management ensures necessary resources to achieve the protection measures documented in the biosecurity plan.

Step 5: Re-evaluate the Institution's Risk Posture and Protection Objectives

- Management regularly reevaluates and makes necessary modifications to the:
 - biosecurity risk statement;
 - biosecurity risk assessment process;
 - the institution's biosecurity program/plan;
 - the institution's biosecurity systems.
- Management assures the daily implementation, training and annual re-evaluation of the security program.

Elements of a Biosecurity Program

Many facilities may determine that existing safety and security programs provide adequate mitigation for the security concerns identified through biosecurity risk assessment. This section offers examples and suggestions for components of a biosecurity program should the risk assessment reveal that further protections may be warranted. Program components should be site-specific and based upon organizational threat/vulnerability assessment and as determined appropriate by facility management. Elements discussed below should be implemented, as needed, based upon the risk assessment process. They should not be construed as "minimum requirements" or "minimum standards" for a biosecurity program.

Program Management

If a biosecurity plan is implemented, institutional management must support the biosecurity program. Appropriate authority must be delegated for implementation and the necessary resources provided to assure program goals are being met. An organizational structure for the biosecurity program that clearly defines the chain of command, roles, and responsibilities should be distributed to the staff. Program management should ensure that biosecurity plans are created, exercised, and revised as needed. The biosecurity program should be integrated into relevant institutional policies and plans.

Physical Security—Access Control and Monitoring

The physical security elements of a laboratory biosecurity program are intended to prevent the removal of assets for non-official purposes. An evaluation of the physical security measures should include a thorough review of the building and premises, the laboratories, and biological material storage areas. Many requirements for a biosecurity plan may already exist in a facility's overall security plan.

Access should be limited to authorized and designated employees based on the need to enter sensitive areas. Methods for limiting access could be as simple as locking doors or having a card key system in place. Evaluations of the levels of access should consider all facets of the laboratory's operations and programs (e.g., laboratory entrance requirements, freezer access). The need for entry by visitors, laboratory workers, management officials, students, cleaning/maintenance staff, and emergency response personnel should be considered.

Personnel Management

Personnel management includes identifying the roles and responsibilities for employees who handle, use, store and transport dangerous pathogens and/or other important assets. The effectiveness of a biosecurity program against identified threats depends, first and foremost, on the integrity of those individuals who have access to pathogens, toxins, sensitive information and/or other assets. Employee screening policies and procedures are used to help evaluate these individuals. Policies should be developed for personnel and visitor identification, visitor management, access procedures, and reporting of security incidents.

Inventory and Accountability

Material accountability procedures should be established to track the inventory, storage, use, transfer and destruction of dangerous biological materials and assets when no longer needed. The objective is to know what agents exist at a facility, where they are located, and who is responsible for them. To achieve this, management should define: 1) the materials (or forms of materials) subject to accountability measures; 2) records to be maintained, update intervals and timelines for record maintenance; 3) operating procedures associated with inventory maintenance (e.g., how material is identified, where it can be used and stored); and 4) documentation and reporting requirements.

It is important to emphasize that microbiological agents are capable of replication and are often expanded to accommodate the nature of the work involving their use. Therefore, knowing the exact "working" quantity of organisms at any given time may be impractical. Depending on the risks associated with a pathogen or toxin, management can designate an individual who is accountable, knowledgeable about the materials in use, and responsible for security of the materials under his or her control.

Information Security

Policies should be established for handling sensitive information associated with the biosecurity program. For the purpose of these policies, “sensitive information” is that which is related to the security of pathogens and toxins, or other critical infrastructure information. Examples of sensitive information may include facility security plans, access control codes, agent inventories and storage locations. Discussion of information security in this section does not pertain to information which has been designated “classified” by the United States pursuant to Executive Order 12958, as amended, and is governed by United States law or to research-related information which is typically unregulated or unrestricted through the peer review and approval processes.

The objective of an information security program is to protect information from unauthorized release and ensure that the appropriate level of confidentiality is preserved. Facilities should develop policies that govern the identification, marking and handling of sensitive information. The information security program should be tailored to meet the needs of the business environment, support the mission of the organization, and mitigate the identified threats. It is critical that access to sensitive information be controlled. Policies for properly identifying and securing sensitive information including electronic files and removable electronic media (e.g., CDs, computer drives) should be developed.

Transport of Biological Agents

Material transport policies should include accountability measures for the movement of materials within an institution (e.g., between laboratories, during shipping and receiving activities) and outside of the facility (e.g., between institutions or locations). Transport policies should address the need for appropriate documentation and material accountability and control procedures for pathogens in transit between locations. Transport security measures should be instituted to ensure that appropriate authorizations have been received and that adequate communication between facilities has occurred before, during, and after transport of pathogens or other potentially hazardous biological materials. Personnel should be adequately trained and familiar with regulatory and institutional procedures for proper containment, packaging, labeling, documentation and transport of biological materials.

Accident, Injury and Incident Response Plans

Laboratory security policies should consider situations that may require emergency responders or public safety personnel to enter the facility in response to an accident, injury or other safety issue or security threat. The preservation of human life, the safety and health of laboratory employees and the surrounding community must take precedence in an emergency over biosecurity concerns. Facilities are encouraged to coordinate with medical, fire, police and other emergency officials when preparing emergency and security breach response

plans. Standard Operation Procedures (SOPs) should be developed that minimize the potential exposure of responding personnel to potentially hazardous biological materials. Laboratory emergency response plans should be integrated with relevant facility-wide or site-specific security plans. These plans should also consider such adverse events as bomb threats, natural disasters and severe weather, power outages, and other facility emergencies that may introduce security threats.

Reporting and Communication

Communication is an important aspect of a biosecurity program. A “chain-of-notification” should be established in advance of an actual event. This communication chain should include laboratory and program officials, institution management, and any relevant regulatory or public authorities. The roles and responsibilities of all involved officials and programs should be clearly defined. Policies should address the reporting and investigation of potential security breaches (e.g., missing biological agents, unusual or threatening phone calls, unauthorized personnel in restricted areas).

Training and Practice Drills

Biosecurity training is essential for the successful implementation of a biosecurity program. Program management should establish training programs that inform and educate individuals regarding their responsibilities within the laboratory and the institution. Practice drills should address a variety of scenarios such as loss or theft of materials, emergency response to accidents and injuries, incident reporting and identification of and response to security breaches. These scenarios may be incorporated into existing emergency response drills such as fire drills or building evacuation drills associated with bomb threats. Incorporating biosecurity measures into existing procedures and response plans often provides efficient use of resources, saves time and can minimize confusion during emergencies.

Security Updates and Re-evaluations

The biosecurity risk assessment and program should be reviewed and updated routinely and following any biosecurity-related incident. Reevaluation is a necessary and on-going process in the dynamic environments of today’s biomedical and research laboratories. Biosecurity program managers should develop and conduct biosecurity program audits and implement corrective actions as needed. Audit results and corrective actions should be documented. The appropriate program officials should maintain records.

Select Agents

If an entity possesses, uses or transfers select agents, it must comply with all requirements of the National Select Agent Program. See Appendix F for

additional guidance on the CDC and USDA Select Agent Programs (42 CFR Part 73; 7 CFR 331 and 9 CFR 121).

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Section VII—Occupational Health and Immunoprophylaxis

The goal of medical support services in a biomedical research setting is to promote a safe and healthy workplace. This is accomplished by limiting opportunities for exposure, promptly detecting and treating exposures, and using information gained from work injuries to further enhance safety precautions. Occupational health and safety in biomedical research settings is a responsibility shared by healthcare providers, safety specialists, principal investigators, employers, and workplace personnel. Optimal worker protection depends on effective, ongoing collaboration among these groups. Supervisors, working with personnel representatives, should describe workers' proposed tasks and responsibilities. First line supervisors and safety professionals should identify the potential worksite health hazards. Principal investigators may serve as subject matter experts. The health provider should design medical support services in consultation with representatives from the institutional environmental health and safety program and the principal investigators. Workers should be fully informed of the available medical support services and encouraged to utilize them. Requisite occupational medical services are described below and expanded discussions of the principles of effective medical support services are available in authoritative texts.^{1,2}

Services offered by the medical support team should be designed to be in compliance with United States Department of Labor (DOL), OSHA regulations, patient confidentiality laws, and the Americans with Disabilities Act of 1990.³⁻⁸ Medical support services should be based upon detailed risk assessments and tailored to meet the organization's needs. Risk assessments should define potential hazards and exposures by job responsibility. They should be provided for all personnel regardless of employment status. Contracted workers, students, and visitors should be provided occupational medical care by their employer or sponsor equivalent to that provided by the host institution for exposures, injuries, or other emergencies experienced at the worksite.

Occupational medical services may be provided through a variety of arrangements (e.g., in-house or community based) as long as the service is readily available and allows timely, appropriate evaluation and treatment. The interaction between worker, healthcare provider and employer may be complex, such as a contract worker who uses his own medical provider or uses contract medical services. Thus, plans for providing medical support for workers should be completed before work actually begins. The medical provider must be knowledgeable about the nature of potential health risks in the work environment and have access to expert consultation.

Prevention is the most effective approach to managing biohazards. Prospective workers should be educated about the biohazards to which they may be occupationally exposed, the types of exposures that place their health at risk, the nature and significance of such risks, as well as the appropriate first aid and follow up for potential exposures. That information should be reinforced

annually, at the time of any significant change in job responsibility, and following recognized and suspected exposures.⁹⁻¹¹

Medical support services for biomedical research facilities should be evaluated annually. Joint annual review of occupational injury and illness reports by healthcare providers and environmental health and safety representatives can assist revision of exposure prevention strategies to minimize occupational health hazards that cannot be eliminated.

Occupational Health Support Service Elements

Preplacement Medical Evaluations

Workers who may be exposed to human pathogens should receive a preplacement medical evaluation. Healthcare providers should be cognizant of potential hazards encountered by the worker. A description of the requirements for the position and an understanding of the potential health hazards present in the work environment, provided by the worker's supervisor, should guide the evaluation. The healthcare provider should review the worker's previous and ongoing medical problems, current medications, allergies to medicines, animals, and other environmental proteins, and prior immunizations. With that information, the healthcare provider determines what medical services are indicated to permit the individual to safely assume the duties of the position. Occasionally, it may be useful to review pre-existing medical records to address specific concerns regarding an individual's medical fitness to perform the duties of a specific position. If pre-existing medical records are unavailable or are inadequate, the healthcare provider may need to perform a targeted medical exam. Comprehensive physical examinations are rarely indicated. During the visit, the healthcare provider should inform the worker of potential health hazards in the work area and review steps that should be taken in the event of an accidental exposure. This visit also establishes a link with the medical support services provider.

When occupational exposure to human pathogens is a risk, employers should consider collecting and storing a serum specimen prior to the initiation of work with the agent. It can be used to establish baseline sero-reactivity, should additional blood samples be collected for serological testing subsequent to a recognized or suspected exposure.

Occasionally, it is desirable to determine an individual's vulnerability to infection with specific agents prior to assigning work responsibilities. Some occupational exposures present substantially more hazard to identifiable sub-populations of workers. Immunodeficient workers or non-immune pregnant female workers may experience devastating consequences from exposures that pose a chance of risk to pregnant women with prior immunity and other immunocompetent workers (e.g., cytomegalovirus or toxoplasmosis). Serologic testing should be used to document baseline vulnerability to specific infections to which the worker might

be exposed, and non-immune workers should be adequately informed about risks. In specific settings, serologic documentation that individual workers have pre-existing immunity to specific infections also may be required for the protection of research animals.¹⁰

Vaccines

Commercial vaccines should be made available to workers to provide protection against infectious agents to which they may be occupationally exposed.¹²⁻¹⁶ The Advisory Committee on Immunization Practices (ACIP) provides expert advice to the Secretary of the DHHS, the Assistant Secretary for Health, and the CDC on the most effective means to prevent vaccine-preventable diseases and to increase the safe usage of vaccines and related biological products. The ACIP develops recommendations for the routine administration of vaccines to pediatric and adult populations, and schedules regarding the appropriate periodicity, dosage, and contraindications. The ACIP is the only entity in the federal government that makes such recommendations. The ACIP is available at the CDC Web site: www.cdc.gov.

If the potential consequences of infection are substantial and the protective benefit from immunization is proven, acceptance of such immunization may be a condition for employment. Current, applicable vaccine information statements must be provided whenever a vaccine is administered. Each worker's immunization history should be evaluated for completeness and currency at the time of employment and re-evaluated when the individual is assigned job responsibilities with a new biohazard.

When occupational exposure to highly pathogenic agents is possible and no commercial vaccine is available, it may be appropriate to immunize workers using vaccines or immune serum preparations that are investigational, or for which the specific indication constitutes an off-label use. Use of investigational products, or of licensed products for off-label indications must be accompanied by adequate informed consent outlining the limited availability of information on safety and efficacy. Use of investigational products should occur through Investigational New Drug (IND) protocols providing safety oversight by both the Food and Drug Administration (FDA) and appropriate Institutional Human Subjects Research Protection Committees.^{17,18} Recommendation of investigational products, as well as commercial vaccines that are less efficacious, associated with high rates of local or systemic reactions, or that produce increasingly severe reactions with repeated use, should be considered carefully. Receipt of such vaccines is rarely justified as a job requirement.

Investigational vaccines for eastern equine encephalomyelitis (EEE) virus, Venezuelan equine encephalitis (VEE) virus, western equine encephalomyelitis (WEE) virus, and Rift Valley fever viruses (RVFV), may be available in limited

quantities and administered on-site at the Special Immunization Program, United States Army Medical Research Institute of Infectious Diseases (USAMRIID).

Periodic Medical Evaluations

Routine, periodic medical evaluations generally are not recommended; however, limited periodic medical evaluations or medical clearances targeted to job requirements may occasionally be warranted (e.g., respirator usage).³ In special circumstances, it may be appropriate to offer periodic laboratory testing to workers with substantial risk of exposure to infectious agents to detect pre-clinical or sub-clinical evidence for an occupationally acquired infection. Before asymptomatic workers without specific exposures are tested for seroreactivity, the benefit of such testing should be justified, plans for further investigation of indeterminate test results should be delineated, and clearly defined criteria for interpretation of results should be developed.

Medical Support for Occupational Illnesses and Injuries

Workers should be encouraged to seek medical evaluation for symptoms that they suspect may be related to infectious agents in their work area, without fear of reprisal. A high index of suspicion for potential occupational exposures should be maintained during any unexplained illness among workers or visitors to worksites containing biohazards. Modes of transmission, as well as the clinical presentation of infections acquired through occupational exposures, may differ markedly from naturally acquired infections. Fatal occupational infections have resulted from apparently trivial exposures. The healthcare provider should have a working understanding of the biohazards present in the workplace and remain alert for subtle evidence of infection and atypical presentations. A close working relationship with the research or clinical program in which the affected employee works is absolutely essential. In the event of injury, consultation between healthcare provider, employee, and the employee's supervisor is required for proper medical management and recordkeeping.

All occupational injuries, including exposures to human pathogens, should be reported to the medical support services provider. Strategies for responding to biohazard exposures should be formulated in advance. Proper post-exposure response is facilitated by exposure-specific protocols that define appropriate first aid, potential post-exposure prophylaxis options, recommended diagnostic tests, and sources of expert medical evaluation. These protocols should address how exposures that occur outside of regular work hours are handled and these protocols should be distributed to potential healthcare providers (e.g., local hospital emergency departments). In exceptional cases, the protocols should be reviewed with state and community public health departments. Emergency medical support training should be provided on a regular basis for both employees and healthcare providers.

The adequacy and timeliness of wound cleansing or other response after an exposure occurs may be the most critical determinant in preventing infection. First aid should be defined, widely promulgated, and immediately available to an injured worker. Barriers to subsequent medical evaluation and treatment should be identified and minimized to facilitate prompt, appropriate care. Laboratory SOPs should include a printed summary of the recommended medical response to specific exposures that can guide immediate response in the work place and that the injured worker can provide to the treating facility. The medical provider's description of the injury should include:

- The potential infectious agent.
- The mechanism and route of exposure (percutaneous, splash to mucous membranes or skin, aerosol, etc.).
- Time and place of the incident.
- Personal protective equipment used at the time of the injury.
- Prior first aid provided (e.g., nature and duration of cleaning and other aid, time that lapsed from exposure to treatment).
- Aspects of the worker's personal medical history relevant to risk of infection or complications of treatment.

First aid should be repeated if the initial adequacy is in question. Healthcare providers must use appropriate barrier precautions to avoid exposure to infectious agents and toxins.

In some instances, it may be possible to prevent or ameliorate illness through post-exposure prophylaxis. Protocols should be developed in advance that clearly identify the situations in which post-exposure prophylaxis are to be considered, the appropriate treatment, and the source of products and expert consultation. Accurate quantification of risk associated with all exposures is not possible, and the decision to administer post-exposure prophylaxis may have to be made quickly and in the absence of confirmatory laboratory testing. Post-exposure regimens may involve off-label use of licensed products (e.g., use of smallpox vaccine for workers exposed to monkeypox) in settings where there is insufficient experience to provide exact guidance on the safety or likely protective efficacy of the prophylactic regimen. Thus, protocols should exist that delineate the circumstances under which it would be appropriate to consider use of each product following exposure, as well as the limits of our understanding of the value of some post-exposure interventions. In these cases, consultations with subject matter experts are especially useful.

Estimating the significance of an exposure may be difficult, despite having established protocols. The clinician may need to make a "best-estimate" based

upon knowledge of similar agents, exposure circumstances, and advice received from knowledgeable experts. Appropriate post-exposure prophylactic response is always pathogen and exposure dependent, and may be host-factor dependent and influenced by immediate post-exposure management. Before prophylactic treatment is undertaken, confirm the likelihood that an exposure occurred, that prophylaxis is indicated and is not contraindicated by past medical history. Conveying this information to the injured worker requires clear, honest communication. The clinical risk assessment and treatment decision process should be carefully explained, the worker's questions addressed with relevant, preprinted educational materials provided. Prompt treatment should be provided, with a mutually agreed plan to follow the individual's clinical course.

The applicable workers compensation claim form should be provided with appropriate explanations for its completion. The supervisor must receive a description of the accident or incident, confirm the circumstances of the injury or exposure and provide relevant advice. The report also should be distributed to all other relevant parties, such as the safety professional. Each incident should receive prompt reconsideration of the initial risk assessment and reevaluation of current strategies to reduce the possibility of future exposures.

Post-exposure serologic testing may be useful, but it is important to determine how information obtained from serologic testing will be interpreted. It is also essential to collect serum specimens at the appropriate interval for a given situation. Assessment of sero-reactivity in exposed workers is most helpful when the results of specimens collected over time can be compared. Ideally specimens collected prior to, at the time of and several weeks following exposure, should be tested simultaneously and results compared to assess changes in the pattern of sero-reactivity. Serum collected too early after exposure may fail to react even when infection has occurred, because antibodies have not yet been produced in detectable quantities. When immediate institution of post-exposure prophylaxis may delay seroconversion, or when the agent to which the worker was exposed results in seroconversion completed over months (e.g., retroviruses), testing of specimens collected late after exposure is particularly important.

Testing of a single serum specimen is generally discouraged and can result in misinterpretation of nonspecific sero-reactivity. Evidence of sero-conversion or a significant (≥ 4 fold) increase in titer associated with a compatible clinical syndrome is highly suggestive of acute infection.

However, the significance of and appropriate response to sero-conversion in the absence of illness is not always clear. If sero-reactivity is evident in the earliest specimen, it is important to re-test that specimen in tandem with serum specimens archived prior to occupational exposure and/or collected serially over time to investigate whether a change in titer suggestive of new infection can be identified.

In some exposure situations, it may be appropriate to store serially collected serum samples, and to send them for testing as evidence of seroconversion only if symptoms develop that suggest an infection may have occurred (e.g., Monkey B virus exposures). Serum collected at the time of employment, and any other specimens not immediately tested should be stored frozen at a temperature of -20° C or lower in a freezer that does not experience freeze-thaw cycles. An inventory system should be established to ensure the accurate and timely retrieval of samples, while protecting patient privacy.

When investigational or other non-commercial assays are utilized, the importance of appropriate controls and the ability to compare serially collected specimens for quantification/characterization of reactivity is increased. The availability of aliquoted samples that allow additional testing may be essential to assist interpretation of ambiguous results. Caution should be taken to avoid placing more confidence in testing outcomes than can be justified by the nature of the assays.

Occupational Health in the BSL-4 Setting

Work with BSL-4 agents involves special challenges for occupational health. Infections of laboratory staff by such agents may be expected to result in serious or lethal disease for which limited treatment options exist. In addition, BSL-4 agents are frequently geographically exotic to the areas in which high containment labs are located but produce immediate public health concern if infections occur in laboratory staff. Potential (if unlikely) transmission from infected staff into the human or animal populations in the areas surrounding the laboratories may raise such concerns to higher levels. Thus, SOPs for BSL-4 settings require special attention to management of unexplained worker absence, including protocols for monitoring, medical evaluation, work-up, and follow-up of workers with unexplained nonspecific illness. Advance planning for the provision of medical care to workers potentially infected with BSL-4 agents is a fundamental component of an occupational health program for a BSL-4 facility.

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Section VIII—Agent Summary Statements

Section VIII-A: Bacterial Agents

Bacillus anthracis

Bacillus anthracis, a gram-positive, non-hemolytic, and non-motile bacillus, is the etiologic agent of anthrax, an acute bacterial disease of mammals, including humans. Like all members of the genus *Bacillus*, under adverse conditions *B. anthracis* has the ability to produce spores that allow the organism to persist for long periods until the return of more favorable conditions. Reports of suspected anthrax outbreaks date back to as early as 1250 BC. The study of anthrax and *B. anthracis* in the 1800s contributed greatly to our general understanding of infectious diseases. Much of Koch's postulates were derived from work on identifying the etiologic agent of anthrax. Louis Pasteur developed the first attenuated live vaccine for anthrax.

Most mammals are susceptible to anthrax; it mostly affects herbivores that ingest spores from contaminated soil and, to a lesser extent, carnivores that scavenge on the carcasses of diseased animals. Anthrax still occurs frequently in parts of central Asia and Africa. In the United States, it occurs sporadically in animals in parts of the West, Midwest and Southwest.

The infectious dose varies greatly from species to species and is route-dependent. The inhalation anthrax infectious dose (ID) for humans primarily has been extrapolated from inhalation challenges of nonhuman primates (NHP) or studies done in contaminated mills. Estimates vary greatly but the medium lethal dose (LD₅₀) is likely within the range of 2,500-55,000 spores.¹ It is believed that very few spores (10 or less) are required for cutaneous anthrax.²

Occupational Infections

Occupational infections are possible when in contact with contaminated animals, animal products or pure cultures of *B. anthracis*, and may include ranchers, veterinarians and laboratory workers. Numerous cases of laboratory-associated anthrax (primarily cutaneous) have been reported.^{3,4} Recent cases include suspected cutaneous anthrax in a laboratory worker in Texas and a cutaneous case in a North Dakota male who disposed of five cows that died of anthrax.^{5,6}

Natural Modes of Infection

The clinical forms of anthrax in humans that result from different routes of infection are: 1) cutaneous (via broken skin); 2) gastrointestinal (via ingestion); and 3) inhalation anthrax. Cutaneous anthrax is the most common and readily treatable form of the disease. Inhalation anthrax used to be known as "Woolsorter disease" due to its prevalence in textile mill workers handling wool and other contaminated animal products. While naturally occurring disease is no longer a

significant public health problem in the United States, anthrax has become a bioterrorism concern. In 2001, 22 people were diagnosed with anthrax acquired from spores sent through the mail, including 11 cases of inhalation anthrax with five deaths and 11 cutaneous cases.⁷

Laboratory Safety and Containment Recommendations

B. anthracis may be present in blood, skin lesion exudates, cerebrospinal fluid, pleural fluid, sputum, and rarely, in urine and feces. The primary hazards to laboratory personnel are: direct and indirect contact of broken skin with cultures and contaminated laboratory surfaces, accidental parenteral inoculation and, rarely, exposure to infectious aerosols. Efforts should be made to avoid production of aerosols by working with infectious organisms in a BSC. In addition, all centrifugation should be done using aerosol-tight rotors that are opened within the BSC after each run.

BSL-2 practices, containment equipment, and facilities are recommended for activities using clinical materials and diagnostic quantities of infectious cultures. ABSL-2 practices, containment equipment and facilities are recommended for studies utilizing experimentally infected laboratory rodents. BSL-3 practices, containment equipment, and facilities are recommended for work involving production quantities or high concentrations of cultures, screening environmental samples (especially powders) from anthrax-contaminated locations, and for activities with a high potential for aerosol production. Workers who frequently centrifuge *B. anthracis* suspensions should use autoclavable aerosol-tight rotors. In addition, regular routine swabbing specimens for culture should be routinely obtained inside the rotor and rotor lid and, if contaminated, rotors should be autoclaved before re-use.

Special Issues

Vaccines A licensed vaccine for anthrax is available. Guidelines for its use in occupational settings are available from the ACIP.^{8,9} Worker vaccination is recommended for activities that present an increased risk for repeated exposures to *B. anthracis* spores including: 1) work involving production quantities with a high potential for aerosol production; 2) handling environmental specimens, especially powders associated with anthrax investigations; 3) performing confirmatory testing for *B. anthracis*, with purified cultures; 4) making repeated entries into known *B. anthracis*-spore-contaminated areas after a terrorist attack; 5) work in other settings in which repeated exposure to aerosolized *B. anthracis* spores might occur. Vaccination is not recommended for workers involved in routine processing of clinical specimens or environmental swabs in general diagnostic laboratories.

Select Agent *B. anthracis* is a select agent requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A Department of Commerce (DoC) permit may be required for the export of this agent to another country. See Appendix C for additional information.

Bordetella pertussis

Bordetella pertussis, an exclusively human respiratory pathogen of worldwide distribution, is the etiologic agent of whooping cough or pertussis. The organism is a fastidious, small gram-negative coccobacillus that requires highly specialized culture and transport media for cultivation in the laboratory. Its natural habitat is the human respiratory tract.

Occupational Infections

Occupational transmission of pertussis has been reported, primarily among healthcare workers.¹⁰⁻¹⁶ Outbreaks, including secondary transmission, among workers have been documented in hospitals, long-term care institutions, and laboratories. Nosocomial transmissions have been reported in healthcare settings. Laboratory-acquired pertussis has been documented.^{17,18}

Natural Modes of Infection

Pertussis is highly communicable, with person-to-person transmission occurring via aerosolized respiratory secretions containing the organism. The attack rate among susceptible hosts is affected by the frequency, proximity, and time of exposure to infected individuals. Although the number of reported pertussis cases declined by over 99% following the introduction of vaccination programs in the 1940s, the 3- to 4-year cycles of cases have continued into the post-vaccination era.¹⁹⁻²¹

Laboratory Safety and Containment Recommendations

The agent may be present in high levels in respiratory secretions, and may be found in other clinical material, such as blood and lung tissue in its infrequent manifestation of septicemia and pneumonia, respectively.^{22,23} Because the natural mode of transmission is via the respiratory route, aerosol generation during the manipulation of cultures and contaminated clinical specimens generates the greatest potential hazard.

BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of known or potentially infectious clinical material and cultures. ABSL-2 practices and containment equipment should be employed for housing experimentally infected animals. Primary containment devices and equipment, including biological safety cabinets, safety centrifuge cups or safety centrifuges should be used for activities likely to

generate potentially infectious aerosols. BSL-3 practices, containment equipment, and facilities are appropriate for production operations.

Special Issues

Vaccines Pertussis vaccines are available but are not currently approved or recommended for use in persons over six years of age. Because this recommendation may change in the near future, the reader is advised to review the current recommendations of the ACIP published in the Morbidity and Mortality Weekly Report (MMWR) and at the CDC Vaccines and Immunizations Web site for the latest recommendations for adolescents and adults.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Brucella species

The genus *Brucella* consists of slow-growing, very small gram-negative coccobacilli whose natural hosts are mammals. Seven *Brucella* species have been described using epidemiologic and biological characteristics, although at the genetic level all brucellae are closely related. *B. melitensis* (natural host: sheep/goats), *B. suis* (natural host: swine), *B. abortus* (natural host: cattle), *B. canis* (natural host: dogs), and *B. "maris"* (natural host: marine mammals) have caused illness in humans exposed to the organism including laboratory personnel.^{24,25} Hypersensitivity to *Brucella* antigens is a potential but rare hazard to laboratory personnel. Occasional hypersensitivity reactions to *Brucella* antigens occur in workers exposed to experimentally and naturally infected animals or their tissues.

Occupational Infections

Brucellosis has been one of the most frequently reported laboratory infections in the past and cases continue to occur.^{4,26-28} Airborne and mucocutaneous exposures can produce LAI. Accidental self-inoculation with vaccine strains is an occupational hazard for veterinarians.

Natural Modes of Infection

Brucellosis (Undulant fever, Malta fever, Mediterranean fever) is a zoonotic disease of worldwide occurrence. Mammals, particularly cattle, goats, swine, and sheep act as reservoirs for brucellae. Multiple routes of transmission have been identified, including direct contact with infected animal tissues or products, ingestion of contaminated milk, and airborne exposure in pens and stables.

Laboratory Safety and Containment Recommendations

Brucella infects the blood and a wide variety of body tissues, including cerebral spinal fluid, semen, pulmonary excretions, placenta, and occasionally urine. Most laboratory-associated cases occur in research facilities and involve exposures to *Brucella* organisms grown in large quantities or exposure to placental tissues containing *Brucella*. Cases have occurred in clinical laboratory settings from sniffing bacteriological cultures²⁹ or working on open bench tops.³⁰ Aerosols from, or direct skin contact with, cultures or with infectious clinical specimens from animals (e.g., blood, body fluids, tissues) are commonly implicated in human infections. Aerosols generated during laboratory procedures have caused multiple cases per exposure.^{30,31} Mouth pipetting, accidental parenteral inoculations, and sprays into eyes, nose and mouth result in infection. The infectious dose of *Brucella* is 10-100 organisms by aerosol route and subcutaneous route in laboratory animals.^{32,33}

BSL-2 practices, containment equipment, and facilities are recommended for routine clinical specimens of human or animal origin. Products of conception containing or believed to contain pathogenic *Brucella* should be handled with BSL-3 practices due to the high concentration of organisms per gram of tissue. BSL-3 and ABSL-3 practices, containment equipment, and facilities are recommended, for all manipulations of cultures of pathogenic *Brucella* spp. listed in this summary, and for experimental animal studies.

Special Issues

Vaccines Human *Brucella* vaccines have been developed and tested in other countries with limited success. A human vaccine is not available in the United States.³⁴

Select Agent *Brucella abortus*, *Brucella melitensis*, and *Brucella suis* are select agents requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Burkholderia mallei

Burkholderia mallei (formerly *Pseudomonas mallei*) is a non-motile gram-negative rod associated with glanders, a rare disease of equine species and humans. While endemic foci of infection exist in some areas of the world, glanders due to natural infection is extremely rare in the United States.

Occupational Infections

Glanders occurs almost exclusively among individuals who work with equine species and/or handle *B. mallei* cultures in the laboratory. *B. mallei* can be very infectious in the laboratory setting. The only reported case of human glanders in the United States over the past 50 years resulted from a laboratory exposure.³⁵ Modes of transmission may include inhalation and/or mucocutaneous exposure.

Natural Mode of Infection

Glanders is a highly communicable disease of horses, goats, and donkeys. Zoonotic transmission occurs to humans, but person-to-person transmission is rare. Clinical glanders no longer occurs in the Western Hemisphere or in most other areas of the world, although enzootic foci are thought to exist in Asia and the eastern Mediterranean.³⁶ Clinical infections in humans are characterized by tissue abscesses and tend to be very serious.

Laboratory Safety and Containment Recommendations

B. mallei can be very hazardous in a laboratory setting. In a pre-biosafety era report, one-half of the workers in a *B. mallei* research laboratory were infected within a year of working with the organism.³⁷ Laboratory-acquired infections have resulted from aerosol and cutaneous exposure.^{37,38} Laboratory infections usually are caused by exposure to bacterial cultures rather than to clinical specimens. Workers should take precautions to avoid exposure to aerosols from bacterial cultures, and to tissues and purulent drainage from victims of this disease.

Primary isolations from patient fluids or tissues may be performed with BSL-2 practices, containment equipment, and facilities in a BSC. Procedures must be performed under BSL-3 containment whenever infectious aerosols or droplets are generated, such as during centrifugation or handling infected animals, or when large quantities of the agent are produced. Procedures conducted outside of a BSC (centrifugation, animal manipulation, etc.) that generate infectious aerosols require respiratory protection. Sealed cups should be used with all centrifuges and these should be opened only inside a BSC. Gloves should be worn when working with potentially infectious material or animals. Animal work with *B. mallei* should be done with ABSL-3 practices, containment equipment, and facilities.

Special Issues

Select Agent *B. mallei* is a select agent requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from

USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Burkholderia pseudomallei

Burkholderia pseudomallei (formerly *Pseudomonas pseudomallei*) is a motile gram-negative, oxidase-positive rod that is found in soil and water environments of equatorial regions, including Southeast Asia, Northern Australia, Central America and South America. This organism is the causative agent of melioidosis, an unusual bacterial disease characterized by abscesses in tissues and organs. Victims of the disease frequently exhibit recrudescence months or years after the initial infection.

Occupational Infections

Melioidosis is generally considered to be a disease associated with agriculture; however, *B. pseudomallei* can be hazardous for laboratory workers. There are two reports of melioidosis in laboratory workers who were infected by aerosols or via skin exposure.^{39,40} Laboratory workers with diabetes are at increased risk of contracting melioidosis.⁴¹

Natural Modes of Infection

While primarily a disease found in Southeast Asia and Northern Australia, melioidosis can occasionally be found in the Americas.⁴² Natural modes of transmission include the exposure of mucous membranes or damaged skin to soil or water containing the organism, the aspiration or ingestion of contaminated water, or the inhalation of dust from contaminated soil. In endemic areas, 5-20% of agricultural workers have antibody titers to *B. pseudomallei*, in the absence of overt disease.⁴³

Laboratory Safety and Containment Recommendations

B. pseudomallei can cause a systemic disease in human patients. Infected tissues and purulent drainage from cutaneous or tissue abscesses can be sources of infection. Blood and sputum also are potential sources of infection.

Work with clinical specimens from patients suspected of having melioidosis and of *B. pseudomallei* cultures may be performed with BSL-2 practices, containment equipment, and facilities. Work should be done in a BSC. Gloves always should be worn when manipulating the microorganism. In cases where infectious aerosols or droplets could be produced, or where production quantities of the organism are generated, these procedures should be confined to BSL-3 facilities with all pertinent primary containment against escape of aerosols. Respiratory protection must be used if the microorganism is manipulated outside of a BSC, such as during centrifugation or handling infected animals. Sealed

cups should be used in all centrifuges and these should be opened only in a BSC. Animal studies with this agent should be done at ABSL-3.

Special Issues

Select Agent *B. pseudomallei* is a select agent requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Campylobacter (C. jejuni subsp. jejuni, C. coli, C. fetus subsp. fetus, C. upsaliensis)

Campylobacters are curved, S-shaped, or spiral rods associated with gastrointestinal infections (primarily *C. jejuni* subsp. *jejuni* and *C. coli*), bacteremia, and sepsis (primarily *C. fetus* subsp. *fetus* and *C. upsaliensis*). Organisms are isolated from stool specimens using selective media, reduced oxygen tension, and elevated incubation temperature (43°C).

Occupational Infections

These organisms rarely cause LAI, although laboratory-associated cases have been documented.⁴⁴⁻⁴⁷ Experimentally infected animals also are a potential source of infection.⁴⁸

Natural Modes of Infection

Numerous domestic and wild animals, including poultry, pets, farm animals, laboratory animals, and wild birds are known reservoirs and are a potential source of infection for laboratory and animal care personnel. While the infective dose is not firmly established, ingestion of as few as 500-800 organisms has caused symptomatic infection.⁴⁹⁻⁵¹ Natural transmission usually occurs from ingestion of organisms in contaminated food or water and from direct contact with infected pets, farm animals, or infants.⁵²

Laboratory Safety and Containment Recommendations

Pathogenic *Campylobacter* sp. may occur in fecal specimens in large numbers. *C. fetus* subsp. *fetus* may also be present in blood, exudates from abscesses, tissues, and sputa. The primary laboratory hazards are ingestion and parenteral inoculation of *C. jejuni*. The significance of aerosol exposure is not known.

BSL-2 practices, containment equipment, and facilities are recommended for activities with cultures or potentially infectious clinical materials. ABSL-2 practices, containment equipment, and facilities are recommended for activities with naturally or experimentally infected animals.

Special Issues

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Chlamydia psittaci (Chlamydophila psittaci), C. trachomatis, C. pneumoniae (Chlamydophila pneumoniae)

Chlamydia psittaci, *C. pneumoniae* (sometimes called *Chlamydophila psittaci* and *Chlamydophila pneumoniae*) and *C. trachomatis* are the three species of *Chlamydia* known to infect humans. Chlamydiae are nonmotile, gram-negative bacterial pathogens with obligate intracellular life cycles. These three species of *Chlamydia* vary in host spectrum, pathogenicity, and in the clinical spectrum of disease. *C. psittaci* is a zoonotic agent that commonly infects psittacine birds and is highly pathogenic for humans. *C. trachomatis* is historically considered an exclusively human pathogen and is the most commonly reported bacterial infection in the United States. *C. pneumoniae* is considered the least pathogenic species, often resulting in subclinical or asymptomatic infections in both animals and humans.

Occupational Infections

Chlamydial infections caused by *C. psittaci* and *C. trachomatis* lymphogranuloma venereum (LGV) strains were at one time among the most commonly reported laboratory-associated bacterial infections.²⁶ In cases reported before 1955⁴, the majority of infections were psittacosis, and these had the highest case fatality rate of laboratory-acquired infectious agents. The major sources of laboratory-associated psittacosis are contact with and exposure to infectious aerosols in the handling, care, or necropsy of naturally or experimentally infected birds. Infected mice and eggs also are important sources of *C. psittaci*. Most reports of laboratory-acquired infections with *C. trachomatis* attribute the infection to inhalation of large quantities of aerosolized organisms during purification or sonification procedures. Early reports commonly attributed infections to exposure to aerosols formed during nasal inoculation of mice or inoculation of egg yolk sacs and harvest of chlamydial elementary bodies. Infections are associated with fever, chills, malaise, and headache; a dry cough is also associated with *C. psittaci* infection. Some workers exposed to *C. trachomatis*

have developed conditions including mediastinal and supraclavicular lymphadenitis, pneumonitis, conjunctivitis, and keratitis.⁵³ Seroconversion to chlamydial antigens is common and often striking although early antibiotic treatment may prevent an antibody response.

Laboratory-associated infections with *C. pneumoniae* have been reported.⁵⁴ Exposed workers were asymptomatic and infection was diagnosed by serology. The route of infection was attributed to inhalation of droplet aerosols created during procedures associated with culture and harvest of the agent from cell culture.

With all species of *Chlamydia*, mucosal tissues in the eyes, nose, and respiratory tract are most often affected by occupational exposures that can lead to infection.

Natural Modes of Infection

C. psittaci is the cause of psittacosis, a respiratory infection that can lead to severe pneumonia requiring intensive care support and possible death. Sequelae include endocarditis, hepatitis, and neurologic complications. Natural infections are acquired by inhaling dried secretions from infected birds. Psittacine birds commonly kept as pets (parrots, parakeets, cockatiels, etc.) and poultry are most frequently involved in transmission. *C. trachomatis* can cause a spectrum of clinical manifestations including genital tract infections, inclusion conjunctivitis, trachoma, pneumonia in infants, and LGV. The LGV strains cause more severe and systemic disease than do genital strains. *C. trachomatis* genital tract infections are sexually transmitted and ocular infections (trachoma) are transmitted by exposure to secretions from infected persons through contact or fomite transmission. *C. pneumoniae* is a common cause of respiratory infection; up to 50% of adults have serologic evidence of previous exposure. Infections with *C. pneumoniae* are transmitted by droplet aerosolization and are most often mild or asymptomatic, although there is a body of evidence associating this agent with chronic diseases such as atherosclerosis and asthma.

Laboratory Safety and Containment Recommendations

C. psittaci may be present in the tissues, feces, nasal secretions and blood of infected birds, and in blood, sputum, and tissues of infected humans. *C. trachomatis* may be present in genital, bubo, and conjunctival fluids of infected humans. Exposure to infectious aerosols and droplets, created during the handling of infected birds and tissues, are the primary hazards to laboratory personnel working with *C. psittaci*. The primary laboratory hazards of *C. trachomatis* and *C. pneumoniae* are accidental parenteral inoculation and direct and indirect exposure of mucous membranes of the eyes, nose, and mouth to genital, bubo, or conjunctival fluids, cell culture materials, and fluids from infected cell cultures or eggs. Infectious aerosols, including those that may be created as a result of centrifuge malfunctions, also pose a risk for infection.

BSL-2 practices, containment equipment, and facilities are recommended for personnel working with clinical specimens and cultures or other materials known or suspected to contain the ocular or genital serovars (A through K) of *C. trachomatis* or *C. pneumoniae*.

BSL-3 practices, containment equipment, and facilities are recommended for activities involving the necropsy of infected birds and the diagnostic examination of tissues or cultures known to contain or be potentially infected with *C. psittaci* strains of avian origin. Wetting the feathers of infected birds with a detergent-disinfectant prior to necropsy can appreciably reduce the risk of aerosols of infected feces and nasal secretions on the feathers and external surfaces of the bird. Activities involving non-avian strains of *C. psittaci* may be performed in a BSL-2 facility as long as BSL-3 practices are followed, including but not limited to the use of primary containment equipment such as BSCs. ABSL-3 practices, containment equipment, and facilities and respiratory protection are recommended for personnel working with naturally or experimentally infected caged birds.

BSL-3 practices and containment equipment are recommended for activities involving work with culture specimens or clinical isolates known to contain or be potentially infected with the LGV serovars (L₁ through L₃) of *C. trachomatis*. Laboratory work with the LGV serovars of *C. trachomatis* can be conducted in a BSL-2 facility as long as BSL-3 practices are followed when handling potentially infectious materials, including but not limited to use of primary containment equipment such as BSCs.

Gloves are recommended for the necropsy of birds and mice, the opening of inoculated eggs, and when there is the likelihood of direct skin contact with infected tissues, bubo fluids, and other clinical materials.

ABSL-2 practices, containment equipment, and facilities are recommended for activities with animals that have been experimentally infected with genital serovars of *C. trachomatis* or *C. pneumoniae*.

BSL-3 practices, containment equipment, and facilities are indicated for activities involving any of these species with high potential for droplet or aerosol production and for activities involving large quantities or concentrations of infectious materials.

Special Issues

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Neurotoxin-producing Clostridia species

Clostridium botulinum, and rare strains of *C. baratii* and *C. butyricum*, are anaerobic spore-forming species that cause botulism, a life-threatening food-borne illness. The pathogenicity of these organisms results from the production of botulinum toxin, one of the most highly potent neurotoxins currently recognized. Purified botulinum neurotoxin is a 150 kDa protein that acts selectively on peripheral cholinergic nerve endings to block neurotransmitter release.⁵⁵ The principal site of action is the neuromuscular junction, where blockade of transmission produces muscle weakness or paralysis. The toxin also acts on autonomic nerve endings where blockade of transmission can produce a variety of adverse effects. The toxin may also contain associated proteins that may increase its size to as high as 900 kDa.

Occupational Infections

There has been only one report of botulism associated with handling of the toxin in a laboratory setting.⁵⁶ However, concerns about potential use of the toxin as an agent of bioterrorism or biological warfare have led to increased handling of the substance by investigators studying mechanism of action and/or developing countermeasures to poisoning.⁵⁷

Natural Modes of Infection

Botulinum toxin occurs in seven different serotypes (A to G), but almost all naturally-occurring human illness is due to serotypes A, B, E, and F.⁵⁸ Botulism occurs when botulinum toxin is released into circulation following ingestion of preformed toxin. However, animal studies have shown that botulism may occur through inhalation of preformed toxin. Use of appropriate personal protective equipment should prevent potential exposure through mucus membranes. Symptoms and even death are possible by accidental injection of botulinum toxin. Risk to toxin exposure is dependent on both route of exposure and toxin molecular weight size. Exposure to neurotoxin producing Clostridia species does not cause infection; however, in certain rare circumstances (Infant Botulism, Wound Botulism, and Adult colonization), the organism can colonize the intestinal tract and other sites and produce toxin. In Wound Botulism, exposure to toxin is caused by introduction of spores into puncture wounds and *in situ* production by the organism. Infants less than 1 year of age may be susceptible to intestinal colonization and develop the syndrome of Infant Botulism as a result of *in situ* production of toxin. Similarly to Infant Botulism, ingestion of spores by adults with a compromised gastrointestinal tract (GI), such as following GI surgery or long-term administration of antibiotics, may increase risk for intestinal infection and *in situ* production of toxin. See the *C. botulinum* Toxin Agent Summary Statement and Appendix I for additional information.

Laboratory Safety and Containment Recommendations

Neurotoxin producing *Clostridia* species or its toxin may be present in a variety of food products, clinical materials (serum, feces) and environmental samples (soil, surface water).⁵⁹ In addition, bacterial cultures may produce very high levels of toxin.⁶⁰ In healthy adults, it is typically the toxin and not the organism that causes disease. Risk of laboratory exposure is due to the presence of the toxin and not due to a potential of infection from the organisms that produce the toxin. Although spore-forming, there is no known risk to spore exposure except for the potential for the presence of residual toxin associated with pure spore preparations. Laboratory safety protocols should be developed with the focus on prevention of accidental exposure to the toxin produced by these *Clostridia* species.

BSL-2 practices, containment equipment, and facilities are recommended for activities that involve the organism or the toxin⁶¹ including the handling of potentially contaminated food. Solutions of sodium hypochlorite (0.1%) or sodium hydroxide (0.1N) readily inactivate the toxin and are recommended for decontamination of work surfaces and for spills. Autoclaving of contaminated materials also is appropriate.

Additional primary containment and personnel precautions, such as those recommended for BSL-3, should be implemented for activities with a high potential for aerosol or droplet production, or for those requiring routine handling of larger quantities of the organism or of the toxin. ABSL-2 practices, containment equipment, and facilities are recommended for diagnostic studies and titration of toxin.

Special Issues

Vaccines A pentavalent (A, B, C, D and E) botulinum toxoid vaccine (PBT) is available through the CDC as an Investigational New Drug (IND). Vaccination is recommended for all personnel working in direct contact with cultures of neurotoxin producing *Clostridia* species or stock solutions of Botulinum neurotoxin. Due to a possible decline in the immunogenicity of available PBT stocks for some toxin serotypes, the immunization schedule for the PBT recently has been modified to require injections at 0, 2, 12, and 24 weeks, followed by a booster at 12 months and annual boosters thereafter. Since there is a possible decline in vaccine efficacy, the current vaccine contains toxoid for only 5 of the 7 toxin types, this vaccine should not be considered as the sole means of protection and should not replace other worker protection measures.

Post-Exposure Treatment An equine antitoxin product is available for treatment of patients with symptoms consistent with botulism. However, due to the risks inherent in equine products, treatment is not provided as a result of exposure unless botulism symptoms are present.

Select Agent Neurotoxin producing *Clostridia* species are select agents requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

***Clostridium tetani* and Tetanus toxin**

Clostridium tetani is an anaerobic endospore-forming gram-positive rod found in the soil and an intestinal tract commensal. It produces a potent neurotoxin, tetanospasmin, which causes tetanus, an acute neurologic condition characterized by painful muscular contractions. Tetanospasmin is an exceedingly potent, high molecular weight protein toxin, consisting of a heavy chain (100kD) subunit that binds the toxin to receptors on neuronal cells and a light chain (50kD) subunit that blocks the release of inhibitory neural transmitter molecules within the central nervous system. The incidence of tetanus in the United States has declined steadily since the introduction of tetanus toxoid vaccines in the 1940's.⁶²

Occupational Infections

Although the risk of infection to laboratory personnel is low, there have been five incidents of laboratory personnel exposure recorded.⁴

Natural Modes of Infection

Contamination of wounds by soil is the usual mechanism of transmission for tetanus. Of the 130 cases of tetanus reported to CDC from 1998 through 2000, acute injury (puncture, laceration, abrasion) was the most frequent predisposing condition. Elevated incidence rates also were observed for persons aged over 60 years, diabetics, and intravenous drug users.⁶³ When introduced into a suitable anaerobic or microaerophilic environment, *C. tetani* spores germinate and produce tetanospasmin. The incubation period ranges from 3 to 21 days. The observed symptoms are primarily associated with the presence of the toxin. Wound cultures are not generally useful for diagnosing tetanus.⁶⁴

Laboratory Safety and Containment Recommendations

The organism may be found in soil, intestinal, or fecal samples. Accidental parenteral inoculation of the toxin is the primary hazard to laboratory personnel. Because it is uncertain if tetanus toxin can be absorbed through mucous membranes, the hazards associated with aerosols and droplets remain unclear.

BSL-2 practices, containment equipment, and facilities are recommended for activities involving the manipulation of cultures or toxin. ABSL-2 practices, containment equipment, and facilities are recommended for animal studies.

Special Issues

Vaccines The vaccination status of workers should be considered in a risk assessment for workers with this organism and/or toxin. While the risk of laboratory-associated tetanus is low, the administration of an adult diphtheria-tetanus toxoid at 10-year intervals further reduces the risk to laboratory and animal care personnel of toxin exposures and wound contamination, and is therefore highly recommended.⁶² The reader is advised to consult the current recommendations of the ACIP.⁶⁵

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Corynebacterium diphtheriae

Corynebacterium diphtheriae is a pleomorphic gram-positive rod that is isolated from the nasopharynx and skin of humans. The organism is easily grown in the laboratory on media containing 5% sheep blood. *C. diphtheriae* produces a potent exotoxin and is the causative agent of diphtheria, one of the most widespread bacterial diseases in the pre-vaccine era.

Occupational Infections

Laboratory-associated infections with *C. diphtheriae* have been documented, but laboratory animal-associated infections have not been reported.^{4,66} Inhalation, accidental parenteral inoculation, and ingestion are the primary laboratory hazards.

Natural Modes of Infection

The agent may be present in exudates or secretions of the nose, throat (tonsil), pharynx, larynx, wounds, in blood, and on the skin. Travel to endemic areas or close contact with persons who have returned recently from such areas, increases risk.⁶⁷ Transmission usually occurs via direct contact with patients or carriers, and more rarely, with articles contaminated with secretions from infected people. Naturally occurring diphtheria is characterized by the development of grayish-white membranous lesions involving the tonsils, pharynx, larynx, or nasal mucosa. Systemic sequelae are associated with the production of diphtheria toxin. An effective vaccine has been developed for diphtheria and this disease has become a rarity in countries with vaccination programs.

Laboratory Safety and Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infected clinical materials or cultures. ABSL-2 facilities are recommended for studies utilizing infected laboratory animals.

Special Issues

Vaccines A licensed vaccine is available. The reader is advised to consult the current recommendations of the CIP.⁶⁵ While the risk of laboratory-associated diphtheria is low, the administration of an adult diphtheria-tetanus toxoid at 10-year intervals may further reduce the risk of illness to laboratory and animal care personnel.⁶²

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Francisella tularensis

Francisella tularensis is a small gram-negative coccobacillus that is carried in numerous animal species, especially rabbits, and is the causal agent of tularemia (Rabbit fever, Deer fly fever, Ohara disease, or Francis disease) in humans. *F. tularensis* can be divided into three subspecies, *F. tularensis* (Type A), *F. holarctica* (Type B) and *F. novicida*, based on virulence testing, 16S sequence, biochemical reactions and epidemiologic features. Type A and Type B strains are highly infectious, requiring only 10-50 organisms to cause disease. Subspecies *F. novicida* is infrequently identified as the cause of human disease. Person-to-person transmission of tularemia has not been documented. The incubation period varies with the virulence of the strain, dose and route of introduction but ranges from 1-4 days with most cases exhibiting symptoms in 3-5 days.⁶⁸

Occupational Infections

Tularemia has been a commonly reported laboratory-associated bacterial infection.⁴ Most cases have occurred at facilities involved in tularemia research; however, cases have been reported in diagnostic laboratories as well. Occasional cases were linked to work with naturally or experimentally infected animals or their ectoparasites.

Natural Modes of Infection

Tick bites, handling or ingesting infectious animal tissues or fluids, ingestion of contaminated water or food and inhalation of infective aerosols are the primary transmission modes in nature. Occasionally, infections have occurred from bites or scratches by carnivores with contaminated mouthparts or claws.

Laboratory Safety and Containment Recommendations

The agent may be present in lesion exudates, respiratory secretions, cerebrospinal fluid (CSF), blood, urine, tissues from infected animals, fluids from infected animals, and fluids from infected arthropods. Direct contact of skin or mucous membranes with infectious materials, accidental parenteral inoculation, ingestion, and exposure to aerosols and infectious droplets has resulted in infection. Infection has been more commonly associated with cultures than with clinical materials and infected animals.⁶⁹

BSL-2 practices, containment equipment, and facilities are recommended for activities involving clinical materials of human or animal origin suspected or known to contain *F. tularensis*. Laboratory personnel should be informed of the possibility of tularemia as a differential diagnosis when samples are submitted for diagnostic tests. BSL-3 and ABSL-3 practices, containment equipment, and facilities are recommended for all manipulations of suspect cultures, animal necropsies and for experimental animal studies. Preparatory work on cultures or contaminated materials for automated identification systems should be performed at BSL-3. Characterized strains of reduced virulence such as *F. tularensis* Type B (strain LVS) and *F. tularensis* subsp *novicida* (strain U112) can be manipulated in BSL-2. Manipulation of reduced virulence strains at high concentrations should be conducted using BSL-3 practices.

Special Issues

Select Agent *F. tularensis* is a select agent requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Helicobacter species

Helicobacters are spiral or curved gram-negative rods isolated from gastrointestinal and hepatobiliary tracts of mammals and birds. There are currently 20 recognized species, including at least nine isolated from humans. Since its discovery in 1982, *Helicobacter pylori* has received increasing attention as an agent of gastritis.⁷⁰ The main habitat of *H. pylori* is the human gastric mucosa. Other *Helicobacter* spp. (*H. cinaedi*, *H. canadensis*, *H. canis*, *H. pullorum*, and *H. fennelliae*) may cause asymptomatic infection as well as proctitis, proctocolitis, enteritis and extraintestinal infections in humans.^{71,72} *H. cinaedi* has been isolated from dogs, cats and Syrian hamsters.

Occupational Infections

Both experimental and accidental LAI with *H. pylori* have been reported.^{73,74} Ingestion is the primary known laboratory hazard. The importance of aerosol exposures is unknown.

Natural Modes of Infection

Chronic gastritis and duodenal ulcers are associated with *H. pylori* infection. Epidemiologic associations have also been made with gastric adenocarcinoma. Human infection with *H. pylori* may be long in duration with few or no symptoms, or may present as an acute gastric illness. Transmission, while incompletely understood, is thought to be by the fecal-oral or oral-oral route.

Laboratory Safety and Containment Recommendations

H. pylori may be present in gastric and oral secretions and stool.⁷⁵ The enterohepatic helicobacters (e.g., *H. canadensis*, *H. canis*, *H. cinaedi*, *H. fennelliae*, *H. pullorum*, and *H. winghamensis*) may be isolated from stool specimens, rectal swabs, and blood cultures.⁷² Protocols involving homogenization or vortexing of gastric specimens have been described for the isolation of *H. pylori*.⁷⁶ Containment of potential aerosols or droplets should be incorporated in these procedures.

BSL-2 practices, containment equipment, and facilities are recommended for activities with clinical materials and cultures known to contain or potentially contain the agents. ABSL-2 practices, containment equipment, and facilities are recommended for activities with experimentally or naturally infected animals.

Special Issues

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Legionella pneumophila and other Legionella-like Agents

Legionella are small, faintly staining gram-negative bacteria. They are obligately aerobic, slow-growing, nonfermentative organisms that have a unique requirement for L-cysteine and iron salts for *in vitro* growth. Legionellae are readily found in natural aquatic bodies and some species (*L. longbeachae*) have been recovered from soil.^{77,78} They are able to colonize hot-water tanks at a temperature range from 40 to 50°C. There are currently 48 known *Legionella* species, 20 of which have been associated with human disease. *L. pneumophila* is the species most frequently encountered in human infections.⁷⁹⁻⁸¹

Occupational Infections

Although laboratory-associated cases of legionellosis have not been reported in the literature, at least one case, due to presumed aerosol or droplet exposure during animal challenge studies with *L. pneumophila*, has been recorded.⁸² Experimental infections have been produced in guinea pigs, mice, rats, embryonated chicken eggs, and human or animal cell lines.⁸³ A fatal case of pneumonia due to *L. pneumophila* was diagnosed in a calf, but only 1.7% (2/112) of the other cattle in the herd had serological evidence of exposure to *Legionella*.⁸⁴ The disease was linked to exposure to a hot water system colonized with *Legionella*. Animal-to-animal transmission has not been demonstrated.

Natural Modes of Infection

Legionella is commonly found in environmental sources, typically in man-made warm water systems. The mode of transmission from these reservoirs is aerosolization, aspiration or direct inoculation into the airway.⁸⁵ Direct person-to-person transmission does not occur. The spectrum of illness caused by *Legionella* species ranges from a mild, self-limited flu-like illness (Pontiac fever) to a disseminated and often fatal disease characterized by pneumonia and respiratory failure (Legionnaires disease). Although rare, *Legionella* has been implicated in cases of sinusitis, cellulitis, pericarditis, and endocarditis.⁸⁶ Legionellosis may be either community-acquired or nosocomial. Risk factors include smoking, chronic lung disease, and immunosuppression. Surgery, especially involving transplantation, has been implicated as a risk factor for nosocomial transmission.

Laboratory Safety and Containment Recommendations

The agent may be present in respiratory tract specimens (sputum, pleural fluid, bronchoscopy specimens, lung tissue), and in extrapulmonary sites. A potential hazard may exist for generation of aerosols containing high concentrations of the agent.

BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of potentially infectious materials, including minimizing the potential for dissemination of the organism from cultures of organisms known to cause disease. ABSL-2 practices, containment equipment and facilities are recommended for activities with experimentally-infected animals. Routine processing of environmental water samples for *Legionella* may be performed with standard BSL-2 practices. For activities likely to produce extensive aerosols and when large quantities of the pathogenic organisms are manipulated, BSL-2 with BSL-3 practices is recommended.

Special Issues

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Leptospira

The genus *Leptospira* is composed of spiral-shaped bacteria with hooked ends. Leptospire are ubiquitous in nature, either free-living in fresh water or associated with renal infection in animals. Historically, these organisms have been classified into pathogenic (*L. interrogans*) and saprophytic (*L. biflexa*) groups, but recent studies have identified more than 12 species based on genetic analysis. These organisms also have been characterized serologically, with more than 200 pathogenic and 60 saprophytic serovars identified as of 2003.⁸⁷ These organisms are the cause of leptospirosis, a zoonotic disease of worldwide distribution. Growth of leptospire in the laboratory requires specialized media and culture techniques, and cases of leptospirosis are usually diagnosed by serology.

Occupational Infections

Leptospirosis is a well-documented laboratory hazard. Approximately, 70 LAI and 10 deaths have been reported.^{4,26} Direct and indirect contact with fluids and tissues of experimentally or naturally infected mammals during handling, care, or necropsy are potential sources of infection.⁸⁸⁻⁹⁰ It is important to remember that rodents are natural carriers of leptospire. Animals with chronic renal infection shed large numbers of leptospire in the urine continuously or intermittently, for long periods of time. Rarely, infection may be transmitted by bites of infected animals.⁸⁸

Natural Modes of Infection

Human leptospirosis typically results from direct contact with infected animals, contaminated animal products, or contaminated water sources. Common routes of infection include abrasions, cuts in the skin or via the conjunctiva. Higher rates of infection observed in agricultural workers and other occupations associated with animal contact.

Laboratory Safety and Containment Recommendations

The organism may be present in urine, blood, and tissues of infected animals and humans. Ingestion, accidental parenteral inoculation, and direct and indirect contact of skin or mucous membranes, particularly the conjunctiva, with cultures or infected tissues or body fluids are the primary laboratory hazards. The importance of aerosol exposure is not known.

BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of known or potentially infective tissues, body fluids, and cultures. The housing and manipulation of infected animals should be performed at ABSL-2. Gloves should be worn to handle and necropsy infected animals and to handle infectious materials and cultures in the laboratory.

Special Issues

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Listeria monocytogenes

Listeria monocytogenes is a gram-positive, non-spore-forming, aerobic bacillus; that is weakly beta-hemolytic on sheep blood agar and catalase-positive.⁹¹ The organism has been isolated from soil, animal feed (silage) and a wide range of human foods and food processing environments. It may also be isolated from symptomatic/asymptomatic animals (particularly ruminants) and humans.^{91,92} This organism is the causative agent of listeriosis, a food-borne disease of humans and animals.

Occupational Infections

Cutaneous listeriosis, characterized by pustular or papular lesions on the arms and hands, has been described in veterinarians and farmers.⁹³ Asymptomatic carriage has been reported in laboratorians.⁹⁴

Natural Modes of Infection

Most human cases of listeriosis result from eating contaminated foods, notably soft cheeses, ready-to-eat meat products (hot dogs, luncheon meats), paté and smoked fish/seafood.⁹⁵ Listeriosis can present in healthy adults with symptoms of fever and gastroenteritis, pregnant women and their fetuses, newborns, and persons with impaired immune function are at greatest risk of developing severe infections including sepsis, meningitis, and fetal demise. In pregnant women, *Listeria monocytogenes* infections occur most often in the third trimester and may precipitate labor. Transplacental transmission of *L. monocytogenes* poses a grave risk to the fetus.⁹²

Laboratory Safety and Containment Recommendations

Listeria monocytogenes may be found in feces, CSF, and blood, as well as numerous food and environmental samples.^{91,92,96,97} Naturally or experimentally infected animals are a source of exposure to laboratory workers, animal care

personnel and other animals. While ingestion is the most common route of exposure, *Listeria* can also cause eye and skin infections following direct contact with the organism.

BSL-2 practices, containment equipment, and facilities are recommended when working with clinical specimens and cultures known or suspected to contain the agent. Gloves and eye protection should be worn while handling infected or potentially infected materials. ABSL-2 practices, containment equipment and facilities are recommended for activities involving experimentally or naturally infected animals. Due to potential risks to the fetus, pregnant women should be advised of the risk of exposure to *L. monocytogenes*.

Special Issues

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Mycobacterium leprae

Mycobacterium leprae is the causative agent of leprosy (Hansen disease). The organism has not been cultivated in laboratory medium but can be maintained in a metabolically active state for some period. Organisms are recovered from infected tissue and can be propagated in laboratory animals, specifically armadillos and the footpads of mice. The infectious dose in humans is unknown. Although naturally occurring leprosy or leprosy-like diseases have been reported in armadillos⁹⁸ and in NHP,^{99,100} humans are the only known important reservoir of this disease.

Occupational Infections

There are no cases reported as a result of working in a laboratory with biopsy or other clinical materials of human or animal origin. However, inadvertent human-to-human transmissions following an accidental needle stick by a surgeon and after use of a presumably contaminated tattoo needle were reported prior to 1950.^{101,102}

Natural Modes of Infection

Leprosy is transmitted from person-to-person following prolonged exposure, presumably via contact with secretions from infected individuals.

Laboratory Safety and Containment Recommendations

The infectious agent may be present in tissues and exudates from lesions of infected humans and experimentally or naturally infected animals. Direct contact of the skin and mucous membranes with infectious materials and accidental parenteral

inoculation are the primary laboratory hazards associated with handling infectious clinical materials. See Appendix B for appropriate tuberculocidal disinfectant.

BSL-2 practices, containment equipment, and facilities are recommended for all activities with known or potentially infectious materials from humans and animals. Extraordinary care should be taken to avoid accidental parenteral inoculation with contaminated sharp instruments. ABSL-2 practices, containment equipment, and facilities are recommended for animal studies utilizing rodents, armadillos, and NHP, because coughing with dissemination of infectious droplets does not occur in these species.

Special Issues

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VIS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Mycobacterium tuberculosis complex

The *Mycobacterium tuberculosis* complex includes *M. tuberculosis*, *M. bovis*, *M. africanum*, and *M. microti* that cause tuberculosis in humans, and more recently recognized *M. caprae* and *M. pinnipedii* that have been isolated from animals. *M. tuberculosis* grows slowly, requiring three weeks for formation of colonies on solid media. The organism has a thick, lipid-rich cell wall that renders bacilli resistant to harsh treatments including alkali and detergents and allows them to stain acid-fast.

Occupational Infections

M. tuberculosis and *M. bovis* infections are a proven hazard to laboratory personnel as well as others who may be exposed to infectious aerosols in the laboratory, autopsy rooms, and other healthcare facilities.^{4,26,103-105} The incidence of tuberculosis in laboratory personnel working with *M. tuberculosis* has been reported to be three times higher than that of those not working with the agent.¹⁰⁶ Naturally or experimentally infected NHP are a proven source of human infection.¹⁰⁷ Experimentally infected guinea pigs or mice do not pose the same hazard because droplet nuclei are not produced by coughing in these species; however, litter from infected animal cages may become contaminated and serve as a source of infectious aerosols.

Natural Modes of Infection

M. tuberculosis is the etiologic agent of tuberculosis, a leading cause of morbidity and mortality worldwide. Persons infected with *M. tuberculosis* can develop active disease within months of infection or can remain latently infected and develop

disease later in life. The primary focus of infection is the lungs, but most other organs can be involved. HIV infection is a serious risk factor for development of active disease. Infectious aerosols produced by coughing spread tuberculosis. *M. bovis* is primarily found in animals but also can produce tuberculosis in humans. It is spread to humans, primarily children, by consumption of non-pasteurized milk and milk products, by handling of infected carcasses, and by inhalation. Human-to-human transmission via aerosols also is possible.

Laboratory Safety and Containment Recommendations

Tubercle bacilli may be present in sputum, gastric lavage fluids, CSF, urine, and in a variety of tissues. Exposure to laboratory-generated aerosols is the most important hazard encountered. Tubercle bacilli may survive in heat-fixed smears¹⁰⁸ and may be aerosolized in the preparation of frozen sections and during manipulation of liquid cultures. Because of the low infective dose of *M. tuberculosis* (i.e., ID₅₀ <10 bacilli), sputa and other clinical specimens from suspected or known cases of tuberculosis must be considered potentially infectious and handled with appropriate precautions. Accidental needle-sticks are also a recognized hazard.

BSL-2 practices and procedures, containment equipment, and facilities are required for non-aerosol-producing manipulations of clinical specimens such as preparation of acid-fast smears. All aerosol-generating activities must be conducted in a BSC. Use of a slide-warming tray, rather than a flame, is recommended for fixation of slides. Liquifaction and concentration of sputa for acid-fast staining may be conducted safely on the open bench by first treating the specimen in a BSC with an equal volume of 5% sodium hypochlorite solution (undiluted household bleach) and waiting 15 minutes before processing.^{109,110}

BSL-3 practices, containment equipment, and facilities are required for laboratory activities in the propagation and manipulation of cultures of any of the subspecies of the *M. tuberculosis* complex and for animal studies using experimentally or naturally infected NHP. Animal studies using guinea pigs or mice can be conducted at ABSL-2.¹¹¹ BSL-3 practices should include the use of respiratory protection and the implementation of specific procedures and use of specialized equipment to prevent and contain aerosols. Disinfectants proven to be tuberculocidal should be used. See Appendix B for additional information.

Manipulation of small quantities of the attenuated vaccine strain *M. bovis* Bacillus Calmette-Guérin (BCG) can be performed at BSL-2 in laboratories that do not culture *M. tuberculosis* and do not have BSL-3 facilities. However, considerable care must be exercised to verify the identity of the strain and to ensure that cultures are not contaminated with virulent *M. tuberculosis* or other *M. bovis* strains. Selection of an appropriate tuberculocidal disinfectant is an important consideration for laboratories working with mycobacteria. See Appendix B for additional information.

Special Issues

Surveillance Annual or semi-annual skin testing with purified protein derivative (PPD) of previously skin-test-negative personnel can be used as a surveillance procedure.

Vaccines The attenuated live BCG, is available and used in other countries but is not used in the United States for immunization.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Mycobacterium spp. other than M. tuberculosis complex and M. leprae

More than 100 species of mycobacteria are recognized. These include both slowly growing and rapidly growing species. In the past, mycobacterial isolates that were not identified as *M. tuberculosis* complex were often called atypical mycobacteria, but these are now more commonly referred to as nontuberculous mycobacteria or mycobacteria other than tuberculosis. Many of the species are common environmental organisms, and approximately 25 of them are associated with infections in humans. A number of additional species are associated with infections in immunocompromised persons, especially HIV-infected individuals. All of these species are considered opportunistic pathogens in humans and none are considered communicable. Mycobacteria are frequently isolated from clinical samples but may not be associated with disease. The most common types of infections and causes are:

1. pulmonary disease with a clinical presentation resembling tuberculosis caused by *M. kansasii*, *M. avium*, and *M. intracellulare*;
2. lymphadenitis associated with *M. avium* and *M. scrofulaceum*;
3. disseminated infections in immunocompromised individuals caused by *M. avium*;
4. skin ulcers and soft tissue wound infections including Buruli ulcer caused by *M. ulcerans*, swimming pool granuloma caused by *M. marinum* associated with exposure to organisms in fresh and salt water and fish tanks, and tissue infections resulting from trauma, surgical procedures, or injection of contaminated materials caused by *M. fortuitum*, *M. chelonae*, and *M. abscessens*.

Occupational Infections

Laboratory-acquired infections with *Mycobacterium* spp. other than *M. tuberculosis* complex have not been reported.

Natural Modes of Infection

Person-to-person transmission has not been demonstrated. Presumably, pulmonary infections are the result of inhalation of aerosolized bacilli, most likely from the surface of contaminated water. Mycobacteria are widely distributed in the environment and in animals. They are also common in potable water supplies, perhaps as the result of the formation of biofilms. The source of *M. avium* infections in immunocompromised persons has not been established.

Laboratory Safety and Containment Recommendations

Various species of mycobacteria may be present in sputa, exudates from lesions, tissues, and in environmental samples. Direct contact of skin or mucous membranes with infectious materials, ingestion, and accidental parenteral inoculation are the primary laboratory hazards associated with clinical materials and cultures. Aerosols created during the manipulation of broth cultures or tissue homogenates of these organisms also pose a potential infection hazard.

BSL-2 practices, containment equipment, and facilities are recommended for activities with clinical materials and cultures of *Mycobacteria* spp. other than *M. tuberculosis* complex. Clinical specimens may also contain *M. tuberculosis* and care must be exercised to ensure the correct identification of cultures. Special caution should be exercised in handling *M. ulcerans* to avoid skin exposure. ABSL-2 practices, containment equipment, and facilities are recommended for animal studies. Selection of an appropriate tuberculocidal disinfectant is an important consideration for laboratories working with mycobacteria. See Appendix B for additional information.

Special Issues

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Neisseria gonorrhoeae

Neisseria gonorrhoeae is a gram-negative, oxidase-positive diplococcus associated with gonorrhea, a sexually transmitted disease of humans. The organism may be isolated from clinical specimens and cultivated in the laboratory using specialized growth media.¹¹²

Occupational Infections

Laboratory-associated gonococcal infections have been reported in the United States and elsewhere.¹¹³⁻¹¹⁶ These infections have presented as conjunctivitis, with either direct finger-to-eye contact or exposure to splashes of either liquid cultures or contaminated solutions proposed as the most likely means of transmission.

Natural Modes of Infection

Gonorrhea is a sexually transmitted disease of worldwide importance. The 2004 rate of reported infections for this disease in the United States was 112 per 100,000 population.¹¹⁷ The natural mode of infection is through direct contact with exudates from mucous membranes of infected individuals. This usually occurs by sexual activity, although newborns may also become infected during birth.¹¹²

Laboratory Safety and Containment Recommendations

The agent may be present in conjunctival, urethral and cervical exudates, synovial fluid, urine, feces, and CSF. Accidental parenteral inoculation and direct or indirect contact of mucous membranes with infectious clinical materials are known primary laboratory hazards. Laboratory-acquired illness due to aerosol transmission has not been documented.

BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of clinical materials or cultures. Gloves should be worn when handling infected laboratory animals and when there is the likelihood of direct skin contact with infectious materials. Additional primary containment and personnel precautions such as those described for BSL-3 may be indicated when there is high risk of aerosol or droplet production, and for activities involving production quantities or high concentrations of infectious materials. Animal studies may be performed at ABSL-2.

Special Issues

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Neisseria meningitidis

Neisseria meningitidis is a gram-negative coccus responsible for serious acute meningitis and septicemia in humans. Virulence is associated with the expression of a polysaccharide capsule. Thirteen different capsular serotypes have been identified, with types A, B, C, Y, and W135 associated with the highest incidence

of disease. The handling of invasive *N. meningitidis* isolates from blood or CSF represents an increased risk to microbiologists.^{118,119}

Occupational Infections

Recent studies of LAI and exposures have indicated that manipulating suspensions of *N. meningitidis* outside a BSC is associated with a high risk for contracting meningococcal disease.¹¹⁹ Investigations of potential laboratory-acquired cases of meningococcal diseases in the United States showed a many-fold higher attack rate for microbiologists compared to that of the United States general population age 30-59 years, and a case fatality rate of 50%, substantially higher than the 12-15% associated with disease among the general population. Almost all the microbiologists had manipulated sterile site isolates on an open laboratory bench.¹²⁰ While isolates obtained from respiratory sources are generally less pathogenic and consequently represent lower risk for microbiologists, rigorous protection from droplets or aerosols is mandated when microbiological procedures are performed on all *N. meningitidis* isolates, especially on those from sterile sites.

Natural Modes of Infection

The human upper respiratory tract is the natural reservoir for *N. meningitidis*. Invasion of organisms from the respiratory mucosa into the circulatory system causes infection that can range in severity from subclinical to fulminant fatal disease. Transmission is person-to-person and is usually mediated by direct contact with respiratory droplets from infected individuals.

Laboratory Safety and Containment Recommendations

N. meningitidis may be present in pharyngeal exudates, CSF, blood, and saliva. Parenteral inoculation, droplet exposure of mucous membranes, infectious aerosol and ingestion are the primary hazards to laboratory personnel. Based on the mechanism of natural infection and the risk associated with handling of isolates on an open laboratory bench, exposure to droplets or aerosols of *N. meningitidis* is the most likely risk for infection in the laboratory.

Specimens for *N. meningitidis* analysis and cultures of *N. meningitidis* not associated with invasive disease may be handled in BSL-2 facilities with rigorous application of BSL-2 standard practices, special practices, and safety equipment. All sterile-site isolates of *N. meningitidis* should be manipulated within a BSC. Isolates of unknown source should be treated as sterile-site isolates.

If a BSC is unavailable, manipulation of these isolates should be minimized, primarily focused on serogroup identification using phenolized saline solution while wearing a laboratory coat, gloves, and safety glasses or full-face splash shield. BSL-3 practices and procedures are indicated for activities with a high potential for droplet or aerosol production and for activities involving production

quantities or high concentrations of infectious materials. Animal studies should be performed under ABSL-2 conditions.

Special Issues

Vaccines The quadrivalent meningococcal polysaccharide vaccine, which includes serogroups A, C, Y, and W-135, will decrease but not eliminate the risk of infection, because it is less than 100% effective and does not provide protection against serogroup B, which caused one-half of the laboratory-acquired cases in the United States in 2000.^{118,120} Laboratorians who are exposed routinely to potential aerosols of *N. meningitidis* should consider vaccination.^{118,121,122}

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Salmonella serotypes, other than S. Typhi

Salmonellae are gram-negative enteric bacteria associated with diarrheal illness in humans. They are motile oxidase-negative organisms that are easily cultivated on standard bacteriologic media, although enrichment and selective media may be required for isolation from clinical materials. Recent taxonomic studies have organized this genus into two species, *S. enterica* and *S. bongori*, containing more than 2500 antigenically distinct subtypes or serotypes.¹²³ *S. enterica* contains the vast majority of serotypes associated with human disease. *S. enterica* serotypes Typhimurium and Enteritidis (commonly designated *S. Typhimurium* and *S. Enteritidis*) are the serotypes most frequently encountered in the United States. This summary statement covers all pathogenic serotypes except *S. Typhi*.

Occupational Infections

Salmonellosis is a documented hazard to laboratory personnel.^{4,26,124-125} Primary reservoir hosts include a broad spectrum of domestic and wild animals, including birds, mammals, and reptiles, all of which may serve as a source of infection to laboratory personnel. Case reports of laboratory-acquired infections indicate a presentation of symptoms (fever, severe diarrhea, abdominal cramping) similar to those of naturally-acquired infections, although one case also developed erythema nodosum and reactive arthritis.^{126,127}

Natural Modes of Infection

Salmonellosis is a food borne disease of worldwide distribution. An estimated 5 million cases of salmonellosis occur annually in the United States. A wide range of domestic and feral animals (poultry, swine, rodents, cattle, iguanas, turtles,

chicks, dogs, cats) may serve as reservoirs for this disease, as well as humans.¹²⁸ The most common mode of transmission is by ingestion of food from contaminated animals or contaminated during processing. The disease usually presents as an acute enterocolitis, with an incubation period ranging from 6 to 72 hours.

Laboratory Safety and Containment Recommendations

The agent may be present in feces, blood, urine, and in food, feed, and environmental materials. Ingestion or parenteral inoculation are the primary laboratory hazards. The importance of aerosol exposure is not known. Naturally or experimentally infected animals are a potential source of infection for laboratory and animal care personnel, and for other animals

Strict compliance with BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials or cultures. This includes conducting procedures with aerosol or high splash potential in primary containment devices such as a BSCs or safety centrifuge cups. Personal protective equipment should be used in accordance with a risk assessment, including splash shields, face protection, gowns, and gloves. The importance of proper gloving techniques and frequent and thorough hand washing is emphasized. Care in manipulating faucet handles to prevent contamination of cleaned hands or the use of sinks equipped with remote water control devices, such as foot pedals, is highly recommended. Special attention to the timely and appropriate decontamination of work surfaces, including potentially contaminated equipment and laboratory fixtures, is strongly advised. ABSL-2 facilities and practices are recommended for activities with experimentally infected animals.

Special Issues

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Salmonella Typhi

Recent taxonomic studies have organized the genus *Salmonella* into two species, *S. enterica* and *S. bongori*, containing more than 2500 antigenically distinct subtypes or serotypes.¹²³ *S. enterica* contains the vast majority of serotypes associated with human disease. *S. enterica* serotype Typhi, commonly designated *S. Typhi*, is the causative agent of typhoid fever. *S. Typhi* is a motile gram-negative enteric bacterium that is easily cultivated on standard bacteriologic media, although enrichment and selective media may be required for isolation of this organism from clinical materials.

Occupational Infections

Typhoid fever is a demonstrated hazard to laboratory personnel.^{4,129,130} Ingestion and less frequently, parenteral inoculation are the most significant modes of transmission in the laboratory. Secondary transmission to other individuals outside of the laboratory is also a concern.¹³¹ Laboratory-acquired *S. Typhi* infections usually present with symptoms of septicemia, headache, abdominal pain, and high fever.¹²⁹

Natural Modes of Infection

Typhoid fever is a serious, potentially lethal bloodstream infection of worldwide distribution. Humans are the sole reservoir and asymptomatic carriers may occur. The infectious dose is low (<10³ organisms) and the incubation period may vary from one to six weeks, depending upon the dose of the organism. The natural mode of transmission is by ingestion of food or water contaminated by feces or urine of patients or asymptomatic carriers.¹²³

Laboratory Safety and Containment Recommendations

The agent may be present in feces, blood, gallbladder (bile), and urine. Humans are the only known reservoir of infection. Ingestion and parenteral inoculation of the organism represent the primary laboratory hazards. The importance of aerosol exposure is not known.

Strict compliance with BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials or cultures. This includes conducting procedures with aerosol or high splash potential in primary containment devices such as BSCs or safety centrifuge cups. Personal protective equipment should be used in accordance with a risk assessment, including splash shields, face protection, gowns, and gloves. The importance of proper gloving techniques and frequent and thorough hand washing is emphasized. Care in manipulating faucet handles to prevent contamination of cleaned hands or the use of sinks equipped with remote water control devices, such as foot pedals, is highly recommended. Special attention to the timely and appropriate decontamination of work surfaces, including potentially contaminated equipment and laboratory fixtures, is strongly advised. BSL-3 practices and equipment are recommended for activities likely to produce significant aerosols or for activities involving production quantities of organisms. ABSL-2 facilities, practices and equipment are recommended for activities with experimentally infected animals. ABSL-3 conditions may be considered for protocols involving aerosols.

Special Issues

Vaccines Vaccines for *S. Typhi* are available and should be considered for personnel regularly working with potentially infectious materials. The reader is advised to consult the current recommendations of the Advisory Committee on

Immunization Practices (ACIP) published in the CDC Morbidity and Mortality Weekly Report for recommendations for vaccination against *S. Typhi*.¹³²

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Shiga toxin (Verocytotoxin)-producing Escherichia coli

Escherichia coli is one of five species in the gram-negative genus *Escherichia*. This organism is a common inhabitant of the bowel flora of healthy humans and other mammals and is one of the most intensively studied prokaryotes. An extensive serotyping system has been developed for *E. coli* based the O (somatic) and H (flagellar) antigens expressed by these organisms. Certain pathogenic clones of *E. coli* may cause urinary tract infections, bacteremia, meningitis, and diarrheal disease in humans, and these clones are associated with specific serotypes.

The diarrheagenic *E. coli* strains have been characterized into at least four basic pathogenicity groups: Shiga toxin (Verocytotoxin)-producing *E. coli* (a subset of which are referred to as enterohemorrhagic *E. coli*), enterotoxigenic *E. coli*, enteropathogenic *E. coli*, and enteroinvasive *E. coli*.¹²³ In addition to clinical significance, *E. coli* strains are commonly-used hosts for cloning experiments and other genetic manipulations in the laboratory. This summary statement provides recommendations for safe manipulation of Shiga toxin-producing *E. coli* strains. Procedures for safely handling laboratory derivatives of *E. coli* or other pathotypes of *E. coli* should be based upon a thorough risk assessment.

Occupational Infections

Shiga toxin-producing *E. coli* strains, including strains of serotype O157:H7, are a demonstrated hazard to laboratory personnel.¹³³⁻¹³⁸ The infectious dose is estimated to be low—similar to that reported for *Shigella* spp., 10-100 organisms.¹³⁶ Domestic farm animals (particularly bovines) are significant reservoirs of the organisms; however, experimentally infected small animals are also sources of infection in the laboratory.¹³⁹ Verocytotoxin-producing *Escherichia coli* have also been in wild birds and rodents in close proximity to farms.¹⁴⁰

Natural Modes of Infection

Cattle represent the most common natural reservoir of Shiga-toxin producing *E. coli*. Transmission usually occurs by ingestion of contaminated food, including raw milk, fruits, vegetables, and particularly ground beef. Human-to-human transmission has been observed in families, day care centers, and custodial institutions. Water-borne transmission has been reported from outbreaks

associated with swimming in a crowded lake and drinking unchlorinated municipal water.¹³⁹ In a small proportion of patients (usually children) infected with these organisms, the disease progresses to hemolytic uremic syndrome or death.

Laboratory Safety and Containment Recommendations

Shiga toxin-producing *E. coli* are usually isolated from feces. However, a variety of food specimens contaminated with the organisms including uncooked ground beef, unpasteurized dairy products and contaminated produce may present laboratory hazards. This agent may be found in blood or urine specimens from infected humans or animals. Accidental ingestion is the primary laboratory hazard. The importance of aerosol exposure is not known.

Strict compliance with BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials or cultures. Procedures with aerosol or high splash potential should be conducted with primary containment equipment or in devices such as a BSC or safety centrifuge cups. Personal protective equipment, such as splash shields, face protection, gowns, and gloves should be used in accordance with a risk assessment. The importance of proper gloving techniques and frequent and thorough hand washing is emphasized. Care in manipulating faucet handles to prevent contamination of cleaned hands or the use of sinks equipped with remote water control devices, such as foot pedals, is highly recommended. Special attention to the timely and appropriate decontamination of work surfaces, including potentially contaminated equipment and laboratory fixtures, is strongly advised. ABSL-2 practices and facilities are recommended for activities with experimentally or naturally infected animals.

Special Issues

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Shigella

The genus *Shigella* is composed of nonmotile gram-negative bacteria in the family Enterobacteriaceae. There are four subgroups that have been historically treated as separate species, even though more recent genetic analysis indicates that they are members of the same species. These include subgroup A (*Shigella dysenteriae*), subgroup B (*S. flexneri*), subgroup C (*S. boydii*), and subgroup D (*S. sonnei*). Members of the genus *Shigella* have been recognized since the late 19th century as causative agents of bacillary dysentery, or shigellosis.¹²³

Occupational Infections

Shigellosis is one of the most frequently reported laboratory-acquired infections in the United States.^{131,141} A survey of 397 laboratories in the United Kingdom revealed that in 1994-1995, four of nine reported laboratory-acquired infections were caused by *Shigella*.¹⁴² Experimentally infected guinea pigs, other rodents, and NHP are proven sources of laboratory-acquired infection.^{143,144}

Natural Modes of Infection

Humans and other large primates are the only natural reservoirs of *Shigella* bacteria. Most transmission is by fecal-oral route; infection also is caused by ingestion of contaminated food or water.¹²³ Infection with *Shigella dysenteriae* type 1 causes more severe, prolonged, and frequently fatal illness than does infection with other *Shigella*. Complications of shigellosis include hemolytic uremic syndrome, which is associated with *S. dysenteriae* 1 infection, and Reiter chronic arthritis syndrome, which is associated with *S. flexneri* infection.

Laboratory Safety and Containment Recommendations

The agent may be present in feces and, rarely, in the blood of infected humans or animals. Accidental ingestion and parenteral inoculation of the agent are the primary laboratory hazards. The 50% infectious dose (oral) of *Shigella* for humans is only a few hundred organisms.¹⁴³ The importance of aerosol exposure is not known.

Strict compliance with BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials or cultures. Procedures with aerosol or high splash potential should be conducted with primary containment equipment such as a BSC or safety centrifuge cups. Personal protective equipment should be used in accordance with a risk assessment, including splash shields, face protection, gowns, and gloves. The importance of proper gloving techniques and frequent and thorough hand washing is emphasized. Care in manipulating faucet handles to prevent contamination of cleaned hands or the use of sinks equipped with remote water control devices, such as foot pedals, is highly recommended. Special attention to the timely and appropriate decontamination of work surfaces, including potentially contaminated equipment and laboratory fixtures, is strongly advised. ABSL-2 facilities and practices are recommended for activities with experimentally or naturally infected animals.

Special Issues

Vaccines Vaccines are currently not available for use in humans.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from

USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Treponema pallidum

Treponema pallidum is a species of extremely fastidious spirochetes that die readily upon desiccation or exposure to atmospheric levels of oxygen, and have not been cultured continuously *in vitro*.¹⁴⁵ *T. pallidum* cells have lipid-rich outer membranes and are highly susceptible to disinfection with common alcohols (i.e., 70% isopropanol). This species contains three subspecies including *T. pallidum* spp. *pallidum* (associated with venereal syphilis), *T. pallidum* spp. *endemicum* (associated with endemic syphilis), and *T. pallidum* spp. *pertenue* (associated with Yaws). These organisms are obligate human pathogens.

Occupational Infections

T. pallidum is a documented hazard to laboratory personnel. Pike lists 20 cases of LAI.⁴ Syphilis has been transmitted to personnel working with a concentrated suspension of *T. pallidum* obtained from an experimental rabbit orchitis.¹⁴⁶ *T. pallidum* is present in the circulation during primary and secondary syphilis. The ID₅₀ of *T. pallidum* needed to infect rabbits by subcutaneous injection has been reported to be as low as 23 organisms.¹⁴⁷ The concentration of *T. pallidum* in patients' blood during early syphilis, however, has not been determined. No cases of laboratory animal-associated infections are reported; however, rabbit-adapted *T. pallidum* (Nichols strain and possibly others) retains virulence for humans.

Natural Modes of Infection

Humans are the only known natural reservoir of *T. pallidum* and transmission occurs via direct sexual contact (venereal syphilis), direct skin contact (Yaws), or direct mucous contact (endemic syphilis). Venereal syphilis is a sexually transmitted disease that occurs in many areas of the world, whereas Yaws occurs in tropical areas of Africa, South America, the Caribbean, and Indonesia. Endemic syphilis is limited to arid areas of Africa and the Middle East.¹⁴⁵

Laboratory Safety and Containment Recommendations

The agent may be present in materials collected from cutaneous and mucosal lesions and in blood. Accidental parenteral inoculation, contact with mucous membranes or broken skin with infectious clinical materials are the primary hazards to laboratory personnel.

BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of blood or other clinical samples from humans or infected rabbits. Gloves should be worn when there is a likelihood

of direct skin contact with infective materials. Periodic serological monitoring should be considered in personnel regularly working with these materials. ABSL-2 practices, containment equipment, and facilities are recommended for work with infected animals.

Special Issues

Vaccines Vaccines are currently not available for use in humans.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Vibrio enteritis species (V. cholerae, V. parahaemolyticus)

Vibrio species are straight or curved motile gram-negative rods. Growth of *Vibrio* species is stimulated by sodium and the natural habitats of these organisms are primarily aquatic environments. Although 12 different *Vibrio* species have been isolated from clinical specimens, *V. cholerae* and *V. parahaemolyticus* are the best-documented causes of human disease.¹⁴⁸ Vibrios may cause either diarrhea or extraintestinal infections.

Occupational Infections

Rare cases of bacterial enteritis due to LAI with either *V. cholerae* or *V. parahaemolyticus* have been reported from around the world.⁴ Naturally and experimentally infected animals¹⁴⁹ and shellfish^{150,151} are potential sources for such illnesses.

Natural Modes of Infection

The most common natural mode of infection is the ingestion of contaminated food or water. The human oral infecting dose of *V. cholerae* in healthy non-achlorhydric individuals is approximately 10^6 - 10^{11} colony forming units,¹⁵² while that of *V. parahaemolyticus* ranges from 10^5 - 10^7 cells.¹⁵³ The importance of aerosol exposure is unknown although it has been implicated in at least one instance.¹⁴⁹ The risk of infection following oral exposure is increased in persons with abnormal gastrointestinal physiology including individuals on antacids, with achlorhydria, or with partial or complete gastrectomies.¹⁵⁴

Laboratory Safety and Containment Recommendations

Pathogenic vibrios can be present in human fecal samples, or in the meats and the exterior surfaces of marine invertebrates such as shellfish. Other clinical specimens from which vibrios may be isolated include blood, arm or leg wounds,

eye, ear, and gallbladder.¹⁴⁸ Accidental oral ingestion of *V. cholerae* or *V. parahaemolyticus* principally results from hands contaminated from the use of syringes or the handling of naturally contaminated marine samples without gloves.

BSL-2 practices, containment equipment, and facilities are recommended for activities with cultures or potentially infectious clinical materials. ABSL-2 practices, containment equipment, and facilities are recommended for activities with naturally or experimentally infected animals.

Special Issues

Vaccines The reader is advised to consult the current recommendations of the ACIP published in the MMWR for vaccination recommendations against *V. cholera*. There are currently no human vaccines against *V. parahaemolyticus*.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Yersinia pestis

Yersinia pestis, the causative agent of plague, is a gram-negative, microaerophilic coccobacillus frequently characterized by a “safety pin” appearance on stained preparations from specimens. It is nonmotile and nonsporulating. There are three biotypes of *Y. pestis*, differentiated by their ability to ferment glycerol and reduce nitrate. All three biotypes are virulent. The incubation period for bubonic plague ranges from two to six days while the incubation period for pneumonic plague is one to six days. Pneumonic plague is transmissible person-to-person;¹⁵⁵ whereas bubonic plague is not. Laboratory animal studies have shown the lethal and infectious doses of *Y. pestis* to be quite low (less than 100 colony forming units).¹⁵⁶

Occupational Infections

Y. pestis is a documented laboratory hazard. Prior to 1950, at least 10 laboratory-acquired cases were reported in the United States, four of which were fatal.^{4,157} Veterinary staff and pet owners have become infected when handling domestic cats with oropharyngeal or pneumonic plague.

Natural Modes of Infection

Infective fleabites are the most common mode of transmission, but direct human contact with infected tissues or body fluids of animals and humans also may serve as sources of infection.

Primary pneumonic plague arises from the inhalation of infectious respiratory droplets or other airborne materials from infected animals or humans. This form of plague has a high case fatality rate if not treated and poses the risk of person-to-person transmission.

Laboratory Safety and Containment Recommendations

The agent has been isolated, in order of frequency of recovery, from bubo aspirate, blood, liver, spleen, sputum, lung, bone marrow, CSF, and infrequently from feces and urine, depending on the clinical form and stage of the disease. Primary hazards to laboratory personnel include direct contact with cultures and infectious materials from humans or animal hosts and inhalation of infectious aerosols or droplets generated during their manipulation. Laboratory and field personnel should be counseled on methods to avoid fleabites and accidental autoinoculation when handling potentially infected live or dead animals.

BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the handling of potentially infectious clinical materials and cultures. In addition, because the infectious dose is so small, all work, including necropsies of potentially infected animals should be performed in a BSC. Special care should be taken to avoid generating aerosols or airborne droplets while handling infectious materials or when performing necropsies on naturally or experimentally infected animals. Gloves should be worn when handling potentially infectious materials including field or laboratory infected animals. BSL-3 is recommended for activities with high potential for droplet or aerosol production, and for activities involving large-scale production or high concentrations of infectious materials. Resistance of *Y. pestis* strains to antibiotics used in the treatment of plague should be considered in a thorough risk assessment and may require additional containment for personal protective equipment. For animal studies, a risk assessment that takes into account the animal species, infective strain, and proposed procedures should be performed in order to determine if ABSL-2 or ABSL-3 practices, containment equipment, and facilities should be employed. BSL-3 facilities and arthropod containment level 3 practices are recommended for all laboratory work involving infected arthropods.¹⁵⁷ See Appendix G for additional information on arthropod containment guidelines.

Special Issues

Select Agent *Yersinia pestis* is an HHS select agent requiring registration with CDC for the possession, use, storage and transfer. See Appendix F for further information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

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Section VIII-B: Fungal Agents

Blastomyces dermatitidis

Blastomyces dermatitidis is a dimorphic fungal pathogen existing in nature and in laboratory cultures at room temperature as a filamentous mold with asexual spores (conidia) that are the infectious particles; these convert to large budding yeasts under the appropriate culture conditions *in vitro* at 37°C and in the parasitic phase *in vivo* in warm-blooded animals. The sexual stage is an Ascomycete with infectious ascospores.

Occupational Infections

Three groups are at greatest risk of laboratory-acquired infection: microbiologists, veterinarians and pathologists.¹ Laboratory-associated local infections have been reported following accidental parenteral inoculation with infected tissues or cultures containing yeast forms of *B. dermatitidis*.²⁻⁸ Pulmonary infections have occurred following the presumed inhalation of conidia from mold-form cultures; two persons developed pneumonia and one had an osteolytic lesion from which *B. dermatitidis* was cultured.^{9,10} Presumably, pulmonary infections are associated only with sporulating mold forms.

Natural Modes of Infection

The fungus has been reported from multiple geographically separated countries, but is best known as a fungus endemic to North America and in association with plant material in the environment. Infections are not communicable, but require common exposure from a point source. Although presumed to dwell within the soil of endemic areas, *B. dermatitidis* is extremely difficult to isolate from soil. Outbreaks associated with the exposure of people to decaying wood have been reported.¹¹

Laboratory Safety and Containment Recommendations

Yeast forms may be present in the tissues of infected animals and in clinical specimens. Parenteral (subcutaneous) inoculation of these materials may cause local skin infection and granulomas. Mold form cultures of *B. dermatitidis* containing infectious conidia, and processing of soil or other environmental samples, may pose a hazard of aerosol exposure.

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for activities with clinical materials, animal tissues, yeast-form cultures, and infected animals. BSL-3 practices, containment equipment, and facilities are required for handling sporulating mold-form cultures already identified as *B. dermatitidis* and soil or other environmental samples known or likely to contain infectious conidia.

Special Issues

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Coccidioides immitis and Coccidioides posadasii

Coccidioides spp. is endemic to lower sonoran deserts of the western hemisphere including northern Mexico, southern Arizona, central and southern California, and west Texas. The original species (*C. immitis*) has been divided into *C. immitis* and *C. posadasii*.¹² These species are dimorphic fungal pathogens existing in nature and in laboratory cultures at room temperature as filamentous molds with asexual spores (single-cell arthroconidia three to five microns in size) that are the infectious particles that convert to spherules under the appropriate culture conditions *in vitro* at 37°C and *in vivo* in warm-blooded animals.

Occupational Infections

Laboratory-associated coccidioidomycosis is a documented hazard of working with sporulating cultures of *Coccidioides* spp.¹³⁻¹⁵ Occupational exposure has also been associated in endemic regions with archeology¹⁶ and high dust exposure.¹⁷ Attack rates for laboratory and occupational exposure are higher than for ambient exposure when large numbers of spores are inhaled. Smith reported that 28 of 31 (90%) laboratory-associated infections in his institution resulted in clinical disease, whereas more than half of infections acquired in nature were asymptomatic.¹⁸ Risk of respiratory infection from exposure to infected tissue or aerosols of infected secretions is very low. Accidental percutaneous inoculation has typically resulted in local granuloma formation.¹⁹

Natural Modes of Infection

Single spores can produce ambient infections by the respiratory route. Peak exposures occur during arid seasons. *Coccidioides* spp. grow in infected tissue as larger multicellular spherules, up to 70 microns in diameter and pose little or no risk of infection from direct exposure.

The majority of ambient infections is subclinical and results in life-long protection from subsequent exposures. The incubation period is one to three weeks and manifests as a community-acquired pneumonia with immunologically mediated fatigue, skin rashes, and joint pain. One of the synonyms for coccidioidomycosis is desert rheumatism. A small proportion of infections is complicated by hematogenous dissemination from the lungs to other organs, most frequently skin, the skeleton, and the meninges. Disseminated infection is

much more likely in persons with cellular immunodeficiencies (AIDS, organ transplant recipient, lymphoma).

Laboratory Safety and Containment Recommendations

Because of their size, the arthroconidia are conducive to ready dispersal in air and retention in the deep pulmonary spaces. The much larger size of the spherule considerably reduces the effectiveness of this form of the fungus as an airborne pathogen.

Spherules of the fungus may be present in clinical specimens and animal tissues, and infectious arthroconidia in mold cultures and soil or other samples from natural sites. Inhalation of arthroconidia from environmental samples or cultures of the mold form is a serious laboratory hazard. Personnel should be aware that infected animal or human clinical specimens or tissues stored or shipped in such a manner as to promote germination of arthroconidia pose a theoretical laboratory hazard.

BSL-2 practices, containment equipment, and facilities are recommended for handling and processing clinical specimens, identifying isolates, and processing animal tissues. ABSL-2 practices, containment equipment, and facilities are recommended for experimental animal studies when the route of challenge is parenteral.

BSL-3 practices, containment equipment, and facilities are recommended for propagating and manipulating sporulating cultures already identified as *Coccidioides* spp. and for processing soil or other environmental materials known to contain infectious arthroconidia. Experimental animal studies should be done at BSL-3 when challenge is via the intranasal or pulmonary route.

Special Issues

Select Agent Some *Coccidioides* spp. are select agents requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Cryptococcus Neoformans

Cryptococcus neoformans is a monomorphic fungal pathogen existing in nature, in laboratory cultures at room temperature and *in vivo* as a budding yeast. The sexual stage is grouped with the Basidiomycetes and is characterized by sparse

hyphal formation with basidiospores. Both basidiospores and asexual yeasts are infectious.

Occupational Infections

Accidental inoculation of a heavy inoculum of *C. neoformans* into the hands of laboratory workers has occurred during injection or necropsy of laboratory animals.^{20,21} Either a local granuloma or no lesion was reported, suggesting low pathogenicity by this route. Respiratory infections as a consequence of laboratory exposure have not been recorded.

Natural Modes of Infection

The fungus is distributed worldwide in the environment and is associated with pigeon feces. Infections are not transmissible from person-to-person, but require common exposure via the respiratory route to a point source.

Laboratory Safety and Containment Recommendations

Accidental parenteral inoculation of cultures or other infectious materials represents a potential hazard to laboratory personnel, particularly to those who may be immunocompromised. Bites by experimentally infected mice and manipulations of infectious environmental materials (e.g., pigeon feces) may also represent a potential hazard to laboratory personnel. *C. neoformans* has been isolated from bedding of cages housing mice with pulmonary infection indicating the potential for contamination of cages and animal facilities by infected animals.²² Reports of cutaneous cryptococcal infection following minor skin injuries suggests that localized infection may complicate skin injuries incurred in laboratories that handle *C. neoformans*.²³

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for activities with known or potentially infectious clinical, environmental, or culture materials and with experimentally infected animals. This agent and any samples that may contain this agent should also be handled in a Class II BSC.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Histoplasma capsulatum

Histoplasma capsulatum is a dimorphic fungal pathogen existing in nature and in laboratory cultures at room temperature as a filamentous mold with asexual spores (conidia); these are the infectious particles that convert to small budding yeasts under the appropriate culture conditions *in vitro* at 37°C

and in the parasitic phase *in vivo*. The sexual stage is an Ascomycete with infectious ascospores.

Occupational Infections

Laboratory-associated histoplasmosis is a documented hazard in facilities conducting diagnostic or investigative work.²⁴⁻²⁷ Pulmonary infections have resulted from handling mold form cultures.^{28,29} Local infection has resulted from skin puncture during autopsy of an infected human,³⁰ from accidental needle inoculation of a viable culture,³¹ and from spray from a needle into the eye.³² Collecting and processing soil samples from endemic areas has caused pulmonary infections in laboratory workers.³³ Conidia are resistant to drying and may remain viable for long periods of time. The small size of the infective conidia (less than 5 microns) is conducive to airborne dispersal and intrapulmonary retention. Work with experimental animals suggests that hyphal fragments are capable of serving as viable inocula.²⁴

Natural Modes of Infection

The fungus is distributed worldwide in the environment and is associated with starling and bat feces. It has been isolated from soil, often in river valleys, between latitudes 45°N and 45°S. Histoplasmosis is naturally acquired by the inhalation of infectious particles, usually microconidia.²⁴ Infections are not transmissible from person-to-person, but require common exposure to a point source.

Laboratory Safety and Containment Recommendations

The infective stage of this dimorphic fungus (conidia) is present in sporulating mold form cultures and in soil from endemic areas. The yeast form in tissues or fluids from infected animals may produce local infection following parenteral inoculation or splash onto mucous membranes.

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for handling and processing clinical specimens, identifying isolates, animal tissues and mold cultures, identifying cultures in routine diagnostic laboratories, and for inoculating experimental animals, regardless of route. Any culture identifying dimorphic fungi should be handled in a Class II BSC.

BSL-3 practices, containment equipment, and facilities are recommended for propagating sporulating cultures of *H. capsulatum* in the mold form, as well as processing soil or other environmental materials known or likely to contain infectious conidia.

Special Issues

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Sporothrix schenckii

Sporothrix schenckii is a dimorphic fungal pathogen existing in nature and in laboratory cultures at room temperature as a filamentous mold with asexual spores (conidia); these are the infectious particles that convert to small budding yeasts in the parasitic phase *in vivo*. The sexual stage is unknown.

Occupational Infections

Most cases of sporotrichosis are reported sporadically following accidental inoculation with contaminated material. Large outbreaks have been documented in persons occupationally or recreationally exposed to soil or plant material containing the fungus. However, *S. schenckii* has caused a substantial number of local skin or eye infections in laboratory personnel.³⁴ Most occupational cases have been associated with accidents and have involved splashing culture material into the eye,^{35,36} scratching,³⁷ or injecting³⁸ infected material into the skin or being bitten by an experimentally infected animal.^{39,40} Skin infections in the absence of trauma have resulted also from handling cultures⁴¹⁻⁴³ or necropsy of animals⁴⁴ without any apparent trauma.

Natural Modes of Infection

The fungus is distributed worldwide in the environment and is associated with sphagnum moss and gardening, often involving sphagnum moss and traumatic implantation. Infections are not transmissible from person-to-person, but require common exposure to a point source. Rare respiratory and zoonotic infections occur. It is thought that naturally occurring lung disease results from inhalation.

Laboratory Safety and Containment Recommendations

Although localized skin and eye infections have occurred in an occupational setting, no pulmonary infections have been reported as a result from laboratory exposure. It should be noted that serious disseminated infections have been reported in immunocompromised persons.⁴⁵

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for laboratory handling of suspected clinical specimens, soil and vegetation, and experimental animal activities with *S. schenckii*. Gloves should

be worn during manipulation of *S. schenckii* and when handling experimentally infected animals. Any culture identifying dimorphic fungi should be handled in a Class II BSC.

Special Issues

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Dermatophytes (Epidermophyton, Microsporum, and Trichophyton)

The dermatophytes are biologically related species of the genera, *Epidermophyton*, *Microsporum*, and *Trichophyton* that exist as monomorphic pathogens in nature, in laboratory cultures at room temperature and *in vivo* as filamentous molds. The sexual stages, when known, are Ascomycetes with infectious ascospores. These fungi are distributed worldwide, with particular species being endemic in particular regions. The species are grouped by natural environment habitat as being primarily associated with humans (anthrophilic), other animals (zoophilic), or soil (geophilic).

Occupational Infections

Although skin, hair, and nail infections by these molds are among the most prevalent of human infections, the processing of clinical material has not been associated with laboratory infections. Infections have been acquired through contacts with naturally or experimentally infected laboratory animals (mice, rabbits, guinea pigs, etc.) and, occasionally, with handling cultures.^{26,29,45,46}

Systemic dermatophytosis is a rare condition. Superficial chronic infections occur frequently among immunocompromised individuals as well as elderly and diabetic persons. Susceptible individuals should use extra caution.⁴⁷⁻⁵⁰

Natural Modes of Infection

Infections can be transmissible from person-to-person, or acquired from common exposure to a point source. The dermatophytes cause infection (dermatophytosis) by invading the keratinized tissues of living animals and are among the most common infectious agents of humans. This fungal group encompasses members of three genera: *Epidermophyton*, *Microsporum*, and *Trichophyton*. The severity of infection depends on the infective species or strain, the anatomic site and other host factors. One of the most severe dermatophytoses is favus, a disfiguring disease of the scalp caused by *Trychophyton schoenleinii*.

Laboratory Safety and Containment Recommendations

Dermatophytes pose a moderate potential hazard to individuals with normal immune status. In the clinical laboratory setting, the inappropriate handling of cultures is the most common source of infection for laboratory personnel. The most common laboratory procedure for detection of the infective dermatophyte is the direct microscopic examination of contaminated skin, hair, and nails, followed by its isolation and identification on appropriated culture media. Direct contact with contaminated skin, hair, and nails of humans could be another source of infection.^{48,49} In research laboratories, dermatophytosis can be acquired by contact with contaminated soil (source of infection: geophilic species) or animal hosts (source of infection: zoophilic species).

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for handling cultures and soil samples. Any culture identifying dimorphic fungi should be handled in a Class II BSC.

Special Issues

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Miscellaneous Molds

Several molds have caused serious infection in immunocompetent hosts following presumed inhalation or accidental subcutaneous inoculation from environmental sources. These agents include the dimorphic mold, *Penicillium marneffe*, and the dematiaceous (brown-pigmented) molds, *Bipolaris* species, *Cladophialophora bantiana*, *Exophiala (Wangiella) dermatitidis*, *Exserohilum* species, *Fonsecaea pedrosoi*, *Ochroconis gallopava (Dactylaria gallopava)*, *Ramichloridium mackenziei (Ramichloridium obovoideum)*, *Rhinocladiella atrovirens*, and *Scedosporium prolificans*.⁵¹

Occupational Infections

Even though no laboratory-acquired infections appear to have been reported with most of these agents, the gravity of naturally-acquired illness is sufficient to merit special precautions in the laboratory. *Penicillium marneffe* has caused a localized infection in a laboratory worker.⁵² It also caused a case of laboratory-acquired disseminated infection following presumed inhalation when an undiagnosed HIV-positive individual visited a laboratory where students were handling cultures on the open bench.⁵³

Natural Modes of Infection

The natural mode of infection varies by specific species; most are poorly characterized.

Laboratory Safety and Containment Recommendations

Inhalation of conidia from sporulating mold cultures or accidental injection into the skin during infection of experimental animals are potential risks to laboratory personnel.

BSL-2 practices, containment equipment, and facilities are recommended for propagating and manipulating cultures known to contain these agents. Any culture identifying dimorphic fungi should be handled in a Class II BSC.

Special Issues

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

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Section VIII-C: Parasitic Agents

General Issues

Additional details about occupationally-acquired cases of parasitic infections, as well as recommendations for post exposure management, are provided elsewhere.¹⁻³ Effective antimicrobial treatment is available for most parasitic infections.⁴ Immunocompromised persons should receive individualized counseling (specific to host and parasite factors) from their personal healthcare provider and their employer about the potential risks associated with working with live organisms.

BSL-2 and ABSL-2 practices,⁵ containment equipment, and facilities are recommended for activities with infective stages of the parasites discussed in this chapter.

Microsporidia, historically considered parasites, are now recognized by most experts to be fungi; however, microsporidia are maintained in the parasitic agent section in this edition. These organisms are discussed here because a laboratory-acquired case of infection has been reported,⁶ and most persons currently still look for microsporidia associated with discussion of parasitic agents.

Importation of parasitic agents may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS.

Blood and Tissue Protozoal Parasites

Blood and tissue protozoal parasites that pose greatest occupational risk include *Babesia*, *Leishmania*, *Plasmodium*, *Toxoplasma*, and *Trypanosoma*. Other tissue protozoa of potential concern include free-living amoeba (*Acanthamoeba*, *Balamuthia mandrillaris*, *Naegleria fowleri*) and some species of microsporidia including *Encephalitozoon cuniculi* that commonly cause extraintestinal infection.

Leishmania spp. cause human leishmaniasis; *Plasmodium* spp. cause human malaria, or some, such as *P. cynomolgi* cause nonhuman primate malaria; *Toxoplasma gondii* causes toxoplasmosis; *Trypanosoma cruzi* causes American trypanosomiasis or Chagas disease; and *Trypanosoma brucei gambiense* and *T. b. rhodesiense* cause African trypanosomiasis or (African) sleeping sickness. With the exception of *Leishmania* and *Toxoplasma*, these agents are classically thought of as bloodborne and have stages that circulate in the blood. Although not always recognized, both *Leishmania* and *Toxoplasma* may have stages that circulate in the blood. Some, such as *Plasmodium* and *Trypanosoma cruzi*, also have tissue stages. *Leishmania* spp. are well recognized to have skin and deep tissue stages and *Toxoplasma gondii* forms tissue cysts, including in the central nervous system.

Occupational Infections

Laboratory-acquired infections with *Leishmania* spp., *Plasmodium* spp., *Toxoplasma gondii*, and *Trypanosoma* spp. have been reported; the majority of these involved needle-stick or other cutaneous exposure to infectious stages of the organisms through abraded skin, including microabrasions.^{1,2}

Laboratory-acquired infections may be asymptomatic. If clinically manifest, they may exhibit features similar to those seen in naturally acquired infections, although bypassing natural modes of infection could result in atypical signs and symptoms. Cutaneous leishmaniasis could manifest as various types of skin lesions (e.g., nodules, ulcers, plaques), while visceral leishmaniasis may result in fever, hepatosplenomegaly, and pancytopenia. However, only one of the laboratorians known to have become infected with *L. (L.) donovani*, an organism typically associated with visceral leishmaniasis, developed clinical manifestations of visceral involvement (e.g., fever, splenomegaly, leukopenia).¹ The other laboratorians developed skin lesions. Laboratory-acquired malaria infections may result in fever and chills, fatigue, and hemolytic anemia. Laboratorians can become infected with *T. gondii* through accidental ingestion of sporulated oocysts, but also may become infected through skin or mucous membrane contact with either tachyzoites or bradyzoites in human or animal tissue or culture. Symptoms in laboratory-acquired *T. gondii* infections may be restricted to flu-like conditions with enlarged lymph nodes, although rash may be present. *Trypanosoma cruzi* infection could manifest initially as swelling and redness at the inoculation site, fever, rash, and adenopathy. Myocarditis and electrocardiographic changes may develop. Infection with *T. b. rhodesiense* and *T. b. gambiense* also may cause initial swelling and redness at the inoculation site, followed by fever, rash, adenopathy, headache, fatigue and neurologic signs.

Blood and tissue protozoal infections associated with exposure to laboratory animals are not common. Potential direct sources of infection for laboratory personnel include accidental needle-stick while inoculating or bleeding animals, contact with lesion material from cutaneous leishmaniasis, and contact with blood of experimentally or naturally infected animals. In the case of rodents experimentally inoculated with *Toxoplasma gondii* via the intraperitoneal route, contact with peritoneal fluid could result in exposure to infectious organisms. Mosquito-transmitted malaria infections can occur under laboratory conditions as nearly half of the occupationally acquired malaria infections were reported to be vector borne, and contact with body fluids (including feces) of reduviids (triatomines) experimentally or naturally infected with *T. cruzi* poses a risk to laboratory personnel.

Babesia microti and other *Babesia* spp. can cause human babesiosis or piroplasmiasis. Under natural conditions, *Babesia* is transmitted by the bite of an infected tick, or by blood transfusion; in the United States, hard ticks (*Ixodes*) are the principal vectors. Although no laboratory infections with *Babesia* have been

reported, they could easily result from accidental needle-stick or other cutaneous exposure of abraded skin to blood containing parasites. Persons who are asplenic, immunocompromised, or elderly have increased risk for severe illness if infected.

Natural Modes of Infection

Leishmaniasis is endemic in parts of the tropics, subtropics, and southern Europe, while malaria is widely distributed throughout the tropics. However, the prevalence of these diseases varies widely among endemic areas; the diseases can be very focal in nature. The four species of malaria that infect humans have no animal reservoir hosts. Some *Leishmania* spp. may have a number of important mammalian reservoir hosts, including rodents and dogs. Only cats and other felines can serve as definitive hosts for *Toxoplasma gondii*, which is distributed worldwide. Birds and mammals, including sheep, pigs, rodents, cattle, deer, and humans can be infected from ingestion of tissue cysts or fecal oocysts and subsequently develop tissue cysts throughout the body. Chagas disease occurs from Mexico southward throughout most of Central and South America, with the exception of the southern-most tip of South America. It has been characterized in some accounts as a zoonotic infection, yet the role of animals in maintaining human infection is unclear. A variety of domestic and wild animals are found naturally infected with *T. cruzi*, but human infection undoubtedly serves as the major source of infection for other humans. African trypanosomiasis is endemic in sub-Saharan Africa but is extremely focal in its distribution. Generally, *T. b. gambiense* occurs in West and Central Africa while *T. b. rhodesiense* occurs in East and Southeast Africa. *T. b. rhodesiense* is a zoonotic infection with cattle or, in a more limited role, game animals serving as reservoir hosts, whereas humans are the only epidemiologically important hosts for *T. b. gambiense*.

Leishmania, *Plasmodium*, and both American and African trypanosomes are all transmitted in nature by blood-sucking insects. Sandflies in the genera *Phlebotomus* and *Lutzomyia* transmit *Leishmania*; mosquitoes in the genus *Anopheles* transmit *Plasmodium*; reduviid (triatomine) bugs such as *Triatoma*, *Rhodnius*, and *Panstrongylus* transmit *T. cruzi* (in the feces rather than the saliva of the bug), and tsetse flies in the genus *Glossina* transmit African trypanosomes.

Laboratory Safety and Containment Recommendations

Infective stages may be present in blood, CSF, bone marrow, or other biopsy tissue, lesion exudates, and infected arthropods. Depending on the parasite, the primary laboratory hazards are skin penetration through wounds or microabrasions, accidental parenteral inoculation, and transmission by arthropod vectors. Aerosol or droplet exposure of organisms to the mucous membranes of the eyes, nose, or mouth are potential hazards when working with cultures of

Leishmania, *Toxoplasma gondii*, or *T. cruzi*, or with tissue homogenates or blood containing hemoflagellates. Immuno-compromised persons should avoid working with live organisms.

Because of the potential for grave consequences of toxoplasmosis in the developing fetus, women who are or might become pregnant and who are at risk for infection with *T. gondii* should receive counseling from their personal physician and employer regarding appropriate means of mitigating the risk (including alternate work assignments, additional PPE, etc.). Working with infectious oocysts poses the greatest risk of acquiring infection; needle-sticks with material containing tachyzoites or bradyzoites also pose a significant risk. Infection with tachyzoites or bradyzoites through mucous membranes or skin abrasions is also possible. Kittens and cats that might be naturally infected with *Toxoplasma* pose some risk to personnel.⁵ Good hygiene and use of personal protection measures would reduce the risk.

One laboratory infection with microsporidia has been reported, associated with conjunctival exposure to spores leading to the development of keratoconjunctivitis. Infection could also result from ingestion of spores in feces, urine, sputum, CSF, or culture. No laboratory-acquired infections have been reported with *Acanthamoeba* spp., *Balamuthia mandrillaris* or *Naegleria fowleri*; however, the possibility of becoming infected by inhalation, by accidental needle-sticks, or through exposure to mucous membranes or microabrasions of the skin should be considered.

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for activities with infective stages of the parasites listed.⁵ Infected arthropods should be maintained in facilities that reasonably preclude the exposure of personnel or the escape of insects. (See Appendix E.) Personal protection (e.g., lab coat, gloves, face shield), in conjunction with containment in a BSC, is indicated when working with cultures, tissue homogenates, or blood containing organisms.

Special Issues

Treatment Highly effective medical treatment for most protozoal infections exists.⁴ An importation or domestic transfer permit for this agent can be obtained from USDA/APHIS/VS.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Intestinal Protozoal Parasites

Intestinal protozoal parasites that pose greatest occupational risk include *Cryptosporidium*, *Isospora*, *Entamoeba histolytica*, and *Giardia*. Other intestinal pathogens of concern are some species of microsporidia, specifically *Septata intestinalis* and *Enterocytozoon bieneusi*. *Cryptosporidium parvum*, *C. hominis*, and *Isospora belli* cause intestinal coccidiosis, most often referred to as cryptosporidiosis and isosporiasis, respectively. *Entamoeba histolytica* can cause both intestinal and extraintestinal infection (e.g., liver abscess) called amebiasis, and *Giardia intestinalis* causes giardiasis.

Occupational Infections

Laboratory-acquired infections with *Cryptosporidium* spp., *E. histolytica*, *G. intestinalis*, and *I. belli* have been reported.¹⁻³ The mode of exposure in laboratory-acquired infections in this group of agents mimics the natural infection routes for the most part, and consequently, clinical symptoms are typically very similar to those seen in naturally acquired infections. For *Cryptosporidium*, *E. histolytica*, *G. intestinalis*, and *I. belli*, the common clinical manifestations are symptoms of gastroenteritis (e.g., diarrhea, abdominal pain and cramping, loss of appetite). Infection with *E. histolytica* may result in bloody stools.

Laboratory animal-associated infections with this group of organisms have been reported and provide a direct source of infection for laboratory personnel who are exposed to feces of experimentally or naturally infected animals.³ Handling *Cryptosporidium* oocysts requires special care, as laboratory-acquired infections have occurred commonly in personnel working with this agent, especially if calves are used as the source of oocysts. Other experimentally infected animals pose potential risks as well. Circumstantial evidence suggests that airborne transmission of oocysts of this small organism (i.e., 4-6 μm diameter) may occur. Rigid adherence to protocol should reduce the occurrence of laboratory-acquired infection in laboratory and animal care personnel.

Natural Modes of Infection

All of these intestinal protozoa have a cosmopolitan distribution, and in some settings, including developed countries, the prevalence of infection can be high. The natural mode of infection for this group of organisms is typically ingestion of an environmentally hardy oocyst (for the coccidia) or cyst (for *E. histolytica* and *G. intestinalis*). The ID_{50} , best established for *Cryptosporidium*, has been shown for some strains to be 5-10 oocysts.⁷ This suggests that even a single oocyst might pose a risk for infection in an exposed laboratorian. The infectious dose for other parasites in this group is not as well established, but is probably in the same range. Further, because these protozoa multiply in the host, ingestion of even small inocula can cause infection and illness. The role for animal reservoir hosts is diverse in this group of organisms. In the case of *C. hominis*, principally humans are infected, whereas for *C. parvum*, humans, cattle, and other

mammals can be infected and serve as reservoir hosts for human infection. In the case of *E. histolytica*, humans serve as the only significant source of infection, and there is no convincing evidence that any animal serves as reservoir host for *I. belli*. The extent to which *Giardia* spp. parasitizing animals can infect humans is only now becoming better understood, but most human infection seems to be acquired from human-to-human transmission. The organisms in this group do not require more than one host to complete their life cycle because they infect, develop, and result in shedding of infectious stages all in a single host. Ingestion of contaminated drinking or recreational water has also been a common source of cryptosporidiosis and giardiasis.

Laboratory Safety and Containment Recommendations

Infective stages may be present in the feces or other body fluids and tissues. Depending on the parasite, ingestion is the primary laboratory hazard. Immunocompromised persons should avoid working with live organisms. Laboratorians who work only with killed or inactivated parasite materials, or parasite fractions, are not at significant risk.

Similarly, no accidental laboratory infection with *Sarcocystis* has been reported, although care should be exercised when working with infected meat products to avoid accidental ingestion. It is not known if laboratorians could be accidentally infected through parenteral inoculation of *Sarcocystis*; nevertheless caution should be exercised when working with cultures, homogenates, etc.

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for activities with infective stages of the parasites listed.⁵ Primary containment (e.g., BSC) or personal protection (e.g., face shield) is especially important when working with *Cryptosporidium*. Oocysts are infectious when shed (i.e., are already sporulated and do not require further development time outside the host), often are present in stool in high numbers, and are environmentally hardy.

Commercially available iodine-containing disinfectants are effective against *E. histolytica* and *G. intestinalis*, when used as directed, as are high concentrations of chlorine (1 cup of full-strength commercial bleach [~5% chlorine] per gallon of water [1:16, vol/vol]).^{1,2}

If a laboratory spill contains *Cryptosporidium* oocysts, the following approach is recommended.² A conventional laboratory detergent/cleaner should be used to remove contaminating matter from surfaces (e.g., of bench tops and equipment). After organic material has been removed, 3% hydrogen peroxide (i.e., undiluted, commercial hydrogen peroxide, identified on the bottle as 3% or "10 vol" hydrogen peroxide) can be used to disinfect surfaces; dispensing bottles that contain undiluted hydrogen peroxide should be readily available in laboratories in which surfaces could become contaminated.

Affected surfaces should be flooded (i.e., completely covered) with hydrogen peroxide. If a large volume of liquid contaminates surfaces, to avoid diluting the hydrogen peroxide, absorb the bulk of the spill with disposable paper towels. Dispense hydrogen peroxide repeatedly, as needed, to keep affected surfaces covered (i.e., wet/moist) for ~30 minutes. Absorb residual hydrogen peroxide with disposable paper towels and allow surfaces to dry thoroughly (10 to 30 minutes) before use. All paper towel litter and other disposable materials should be autoclaved or similarly disinfected before disposal. Reusable laboratory items can be disinfected and washed in a laboratory dishwasher by using the “sanitize” cycle and a detergent containing chlorine. Alternatively, immerse contaminated items for ~1 hour in a water bath preheated to 50° C; thereafter, wash them in a detergent/disinfectant solution.

Special Issues

Treatment Highly effective medical treatment exists for most protozoal infections; treatment with nitazoxanide for *Cryptosporidium* is now available, but efficacy has not been proven.⁴

Transfer of Agent Importation of these agents may require CDC and/or USDA importation permits. Domestic transport of these agents may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Trematode Parasites

Trematode parasites that pose greatest occupational risk are the *Schistosoma* spp., although others including *Fasciola* are of concern. *Schistosoma mansoni* causes intestinal schistosomiasis or bilharziasis, also known as Manson’s blood fluke, in which the adult flukes reside in the venules of the bowel and rectum. *Fasciola hepatica*, the sheep liver fluke, causes fascioliasis, where the adult flukes live in the common and hepatic bile ducts of the human or animal host.

Occupational Infections

Laboratory-acquired infections with *S. mansoni* and *F. hepatica* have been reported, but accidental infections with other *Schistosoma* spp. could also occur.^{1,2} By nature of the infection, none have been directly associated with laboratory animals, with the exception of infected mollusk intermediate hosts.

Laboratory-acquired infections with *F. hepatica* may be asymptomatic, but could have clinical manifestations such as right upper quadrant pain, biliary colic, obstructive jaundice, elevated transaminase levels, and other pathology associated with hepatic damage resulting from migration of the fluke through the liver en route to the bile duct. Most laboratory exposures to schistosomes would

result in predictably low worm burdens with minimal disease potential. However, clinical manifestations of infection with *S. mansoni* could include dermatitis, fever, cough, hepatosplenomegaly, and adenopathy.

Natural Modes of Infection

Fasciola hepatica has a cosmopolitan distribution and is most common in sheep-raising areas, although other natural hosts include goats, cattle, hogs, deer, and rodents. Snails in the family Lymnaeidae, primarily species of *Lymnaea*, are intermediate hosts for *F. hepatica*, and release cercariae that encyst on vegetation. Persons become infected with *F. hepatica* by eating raw or poorly cooked vegetation, especially green leafy plants such as watercress, on which metacercariae have encysted.

Schistosoma mansoni is widely distributed in Africa, South America, and the Caribbean; the prevalence of infection has been rapidly changing in some areas. Infection occurs when persons are exposed to free-swimming cercariae in contaminated bodies of water; cercariae can penetrate intact skin. The natural snail hosts capable of supporting development of *S. mansoni* are various species of *Biomphalaria*.

Laboratory Safety and Containment Recommendations

Infective stages of *F. hepatica* (metacercariae) and *S. mansoni* (cercariae) may be found, respectively, encysted on aquatic plants or in the water in laboratory aquaria used to maintain snail intermediate hosts. Ingestion of fluke metacercariae and skin penetration by schistosome cercariae are the primary laboratory hazards. Dissection or crushing of schistosome-infected snails may also result in exposure of skin or mucous membrane to cercariae-containing droplets. Additionally, metacercariae may be inadvertently transferred from hand to mouth by fingers or gloves, following contact with contaminated aquatic vegetation or aquaria.

All reported cases of laboratory-acquired schistosomiasis have been caused by *S. mansoni*, which probably reflects the fact that many more laboratories work with *S. mansoni* than with other *Schistosoma* spp. However, accidental infection with *S. haematobium*, *S. japonicum*, and *S. mekongi* could easily occur in the same manner as described for *S. mansoni*.

Exposure to cercariae of non-human species of schistosomes (e.g., avian species) may cause mild to severe dermatitis (swimmer's itch).

BSL-2 and ABSL-2 practices, containment equipment and facilities are recommended for laboratory work with infective stages of the parasites listed.⁵ Gloves should be worn when there may be direct contact with water containing cercariae or vegetation with encysted metacercariae from naturally or experimentally infected snail intermediate hosts. Long-sleeved laboratory coats or other protective garb should be worn when working in the immediate area of

aquaria or other water sources that may contain schistosome cercariae. Water from laboratory aquaria containing snails and cercariae should be decontaminated (e.g., ethanol, hypochlorite, iodine, or heat) before discharged to sanitary sewers.

Special Issues

Treatment Highly effective medical treatment for most trematode infections exists.⁴

Transfer of Agent Importation of these agents may require CDC and/or USDA importation permits. Domestic transport of these agents may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Cestode Parasites

Cestode parasites of potential risk for laboratorians include *Echinococcus* spp., *Hymenolepis nana*, and *Taenia solium*. Echinococcosis is an infection caused by cestodes in the genus *Echinococcus*; *E. granulosus* causes cystic echinococcosis, *E. multilocularis* causes alveolar echinococcosis, and *E. vogeli* and *E. oligarthrus* cause polycystic echinococcosis. Humans serve as intermediate hosts and harbor the metacestode or larval stage, which produces a hydatid cyst. *Hymenolepis nana*, the dwarf tapeworm, is cosmopolitan in distribution and produces hymenolepiasis, or intestinal infection with the adult tapeworm. *Taenia solium*, the pork tapeworm, causes both taeniasis (infection of the intestinal tract with the adult worm), and cysticercosis (infection of subcutaneous, intermuscular, and central nervous system with the metacestode stage or cysticercus).

Occupational Infections

No laboratory-acquired infections have been reported with any cestode parasite.

Natural Modes of Infection

The infectious stage of *Echinococcus*, *Hymenolepis*, and *Taenia* is the oncosphere contained within the egg. *Hymenolepis nana* is a one-host parasite and does not require an intermediate host; it is directly transmissible by ingestion of feces of infected humans or rodents. The life cycles of *Echinococcus* and *Taenia* require two hosts. Canids, including dogs, wolves, foxes, coyotes, and jackals, are the definitive hosts for *E. granulosus*, and various herbivores such as sheep, cattle, deer, and horses are the intermediate hosts. Foxes and coyotes are the principal definitive hosts for *E. multilocularis*, although dogs and cats also can become infected and rodents serve as the intermediate hosts. Bush dogs and pacas serve as the definitive and intermediate hosts, respectively, for *E. vogeli*. Dogs also may be infected. *Echinococcus oligarthrus* uses wild felines,

including cougar, jaguarondi, jaguar, ocelot, and pampas cat, as definitive hosts and various rodents such as agoutis, pacas, spiny rats, and rabbits serve as intermediate hosts. People become infected when eggs shed by the definitive host are accidentally ingested. For *T. solium*, people can serve both as definitive host (harbor the adult tapeworm), and as accidental intermediate host (harbor the larval stages cysticerci). Pigs are the usual intermediate host, becoming infected as they scavenge human feces containing eggs.

Laboratory Safety and Containment Recommendations

Infective eggs of *Echinococcus* spp. may be present in the feces of carnivore definitive hosts.³ *Echinococcus granulosus* poses the greatest risk because it is the most common and widely distributed species, and because dogs are the primary definitive hosts. For *T. solium*, infective eggs in the feces of humans serve as the source of infection. Accidental ingestion of infective eggs from these sources is the primary laboratory hazard. Ingestion of cysticerci of *T. solium* (*Cysticercus cellulosae*) leads to human infection with the adult tapeworm. For those cestodes listed, the ingestion of a single infective egg from the feces of the definitive host could potentially result in serious disease. Ingestion of the eggs of *H. nana* in the feces of definitive hosts (humans or rodents) could result in intestinal infection.

Although no laboratory-acquired infections with either *Echinococcus* spp. or *T. solium* have been reported, the consequences of such infections could be serious. Laboratory-acquired infections with cestodes could result in various clinical manifestations, depending upon the type of cestode. Human infection with *Echinococcus* spp. could range from asymptomatic to severe. The severity and nature of the signs and symptoms depends upon the location of the cysts, their size, and condition (alive versus dead). Clinical manifestations of a liver cyst could include hepatosplenomegaly, right epigastric pain, and nausea, while a lung cyst may cause chest pain, dyspnea, and hemoptysis. For *T. solium*, ingestion of eggs from human feces can result in cysticercosis, with cysts located in subcutaneous and intermuscular tissues, where they may be asymptomatic. Cysts in the central nervous system may cause seizures and other neurologic symptoms. Ingestion of tissue cysts of *T. solium* can lead to development of adult worms in the intestine of humans. Immunocompromised persons working with these cestodes must take special care as the asexual multiplication of the larval stages of these parasites makes them especially dangerous to such persons.

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for work with infective stages of these parasites.⁵ Special attention should be given to personal hygiene (e.g., hand washing) and laboratory practices that would reduce the risk of accidental ingestion of infective eggs. Gloves are recommended when there may be direct contact with feces or with surfaces contaminated with fresh feces of carnivores infected with *Echinococcus* spp., humans infected with *T. solium*, or humans or rodents infected with *H. nana*.

Special Issues

Treatment Highly effective medical treatment for most cestode infections exists.⁴

Transfer of Agent Importation of these agents may require CDC and/or USDA importation permits. Domestic transport of these agents may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Nematode Parasites

Nematode parasites that pose greatest occupational risk include the ascarids, especially *Ascaris* and *Baylisascaris*; hookworms, both human and animal; *Strongyloides*, both human and animal; *Enterobius*; and the human filariae, primarily *Wuchereria* and *Brugia*. *Ancylostoma braziliense* and *A. caninum* cause hookworm infection in cats and dogs, respectively. *Ascaris lumbricoides* causes ascariasis and is known as the large intestinal roundworm of humans. *Enterobius vermicularis*, known as the human pinworm or seatworm, causes enterobiasis or oxyuriasis. *Strongyloides*, the threadworm, causes strongyloidiasis. *Ancylostoma*, *Ascaris*, and *Strongyloides* reside as adults in the small intestine of their natural hosts, whereas *E. vermicularis* colonizes the cecum and appendix.

Occupational Infections

Laboratory-associated infections with *Ancylostoma* spp., *A. lumbricoides*, *E. vermicularis*, and *Strongyloides* spp. have been reported.¹⁻³ Laboratory infections with hookworms and *Strongyloides* presumptively acquired from infected animals have been reported. Allergic reactions to various antigenic components of human and animal ascarids (e.g., aerosolized antigens) may pose risk to sensitized persons.

Laboratory-acquired infections with these nematodes can be asymptomatic, or can present with a range of clinical manifestations dependent upon the species and their location in host. Infection with hookworm of animal origin can result in cutaneous larva migrans or creeping eruption of the skin. Infection with *A. lumbricoides* may produce cough, fever, and pneumonitis as larvae migrate through the lung, followed by abdominal cramps and diarrhea or constipation from adult worms in the intestine. Infection with *E. vermicularis* usually causes perianal pruritis, with intense itching. Infection with animal *Strongyloides* spp. may induce cutaneous larva migrans.

Natural Modes of Infection

Ancylostoma infection in dogs and cats is endemic worldwide. Human infection occurs through penetration of the skin. Cutaneous larva migrans or creeping eruption occurs when infective larvae of animal hookworms, typically dog and

cat hookworms, penetrate the skin and begin wandering. *Ancylostoma* larvae can also cause infection if ingested. These larvae do not typically reach the intestinal tract, although *A. caninum* has on rare occasions developed into non-gravid adult worms in the human gut.

Ascaris lumbricoides infection is endemic in tropical and subtropical regions of the world. Infection occurs following accidental ingestion of infective eggs. Unembryonated eggs passed in the stool require two to three weeks to become infectious, and *Ascaris* eggs are very hardy in the environment.

Enterobius vermicularis occurs worldwide, although infection tends to be more common in school-age children than adults, and in temperate than tropical regions. Pinworm infection is acquired by ingestion of infective eggs, most often on contaminated fingers following scratching of the perianal skin. Eggs passed by female worms are not immediately infective, but only require several hours' incubation to become fully infectious. Infection with this worm is relatively short (60 days on average), and reinfection is required to maintain an infection.

Strongyloides infection in animals is endemic worldwide. People become infected with animal *Strongyloides* when infective, filariform larvae penetrate the skin, and can develop cutaneous creeping eruption (larva currens).

Laboratory Safety and Containment Recommendations

Eggs and larvae of most nematodes are not infective in freshly passed feces; development to the infective stages may require from one day to several weeks. Ingestion of the infective eggs or skin penetration by infective larvae are the primary hazards to laboratory staff and animal care personnel. Development of hypersensitivity is common in laboratory personnel with frequent exposure to aerosolized antigens of ascarids.

Ascarid eggs are sticky, and special care should be taken to ensure thorough cleaning of contaminated surfaces and equipment. Caution should be used even when working with formalin-fixed stool samples because ascarid eggs can remain viable and continue to develop to the infective stage in formalin.⁸

Working with infective eggs of other ascarids, such as *Toxocara* and *Baylisascaris*, poses significant risk because of the potential for visceral migration of larvae, including invasion of the eyes and central nervous system. *Strongyloides stercoralis* is of particular concern to immuno-suppressed persons because potentially life-threatening systemic hyperinfection can occur. Lugol's iodine kills infective larvae and should be sprayed onto skin or laboratory surfaces that are contaminated accidentally. The larvae of *Trichinella* in fresh or digested tissue could cause infection if accidentally ingested. Arthropods infected with filarial parasites pose a potential hazard to laboratory personnel.

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for activities with infective stages of the nematodes listed here.⁵ Exposure to aerosolized sensitizing antigens of ascarids should be avoided. Primary containment (e.g., BSC) is recommended for work that may result in aerosolization of sensitization from occurring.

Special Issues

Treatment Highly effective medical treatment for most nematode infections exists.⁴

Transfer of Agent Importation of these agents may require CDC and/or USDA importation permits. Domestic transport of these agents may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

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Section VIII-D: Rickettsial Agents

Coxiella burnetii

Coxiella burnetii is the etiologic agent of Q fever. *C. burnetii* is a bacterial obligate intracellular pathogen that undergoes its developmental cycle within an acidic vacuolar compartment exhibiting many characteristics of a phagolysosome. The developmental cycle consists of a large (approximately 1 µm in length) cell variant that is believed to be the more metabolically active, replicative cell type and a smaller, more structurally stable cell variant that is highly infectious and quite resistant to drying and environmental conditions.¹⁻⁴ The organism undergoes a virulent (Phase I) to avirulent (Phase II) transition upon serial laboratory passage in eggs or tissue culture.

The infectious dose of virulent Phase I organisms in laboratory animals has been calculated to be as small as a single organism.⁵ The estimated human infectious dose for Q fever by inhalation is approximately 10 organisms.⁶ Typically, the disease manifests with flu-like symptoms including fever, headache, and myalgia but can also cause pneumonia and hepatomegaly. Infections range from sub-clinical to severe although primary infections respond readily to antibiotic treatment. Although rare, *C. burnetii* is known to cause chronic infections such as endocarditis or granulomatous hepatitis.⁷

Occupational Infections

Q fever is the second most commonly reported LAI in Pike's compilation. Outbreaks involving 15 or more persons were recorded in several institutions.^{8,9} Infectious aerosols are the most likely route of laboratory-acquired infections. Experimentally infected animals also may serve as potential sources of infection or laboratory and animal care personnel. Exposure to naturally infected, often asymptomatic sheep and their birth products is a documented hazard to personnel.^{10,11}

Natural Modes of Infection

Q fever (Q for query) occurs worldwide. Broad ranges of domestic and wild mammals are natural hosts for Q fever and sources of human infection. Parturient animals and their birth products are common sources of infection. The placenta of infected sheep may contain as many as 10⁹ organisms per gram of tissue¹² and milk may contain 10⁵ organisms per gram. The resistance of the organism to drying and its low infectious dose can lead to dispersal from contaminated sites.

Laboratory Safety and Containment Recommendations

The necessity of using embryonated eggs or cell culture techniques for the propagation of *C. burnetii* leads to extensive purification procedures. Exposure to infectious aerosols and parenteral inoculation cause most infections in laboratory and animal care personnel.^{8,9} The agent may be present in infected arthropods

and in the blood, urine, feces, milk, and tissues of infected animals or human hosts. Exposure to naturally infected, often asymptomatic, sheep and their birth products is a documented hazard to personnel.^{10,11} Recommended precautions for facilities using sheep as experimental animals are described elsewhere.^{10,13}

BSL-2 practices and facilities are recommended for nonpropagative laboratory procedures, including serological examinations and staining of impression smears. BSL-3 practices and facilities are recommended for activities involving the inoculation, incubation, and harvesting of embryonated eggs or cell cultures, the necropsy of infected animals and the manipulation of infected tissues. Experimentally infected animals should be maintained under ABSL-3 because infected rodents may shed the organisms in urine or feces.⁸ A specific plaque-purified clonal isolate of an avirulent (Phase II) strain (Nine Mile) may be safely handled under BSL-2 conditions.¹⁴

Special Issues

Vaccines An investigational Phase I, Q fever vaccine (IND) is available on a limited basis from the Special Immunizations Program (301-619-4653) of the USAMRIID, Fort Detrick, Maryland, for at-risk personnel under a cooperative agreement with the individual's requesting institution. The use of this vaccine should be restricted to those who are at high risk of exposure and who have no demonstrated sensitivity to Q fever antigen. The vaccine can be reactogenic in those with prior immunity, thus requires skin testing before administration. The vaccine is only administered at USAMRIID and requires enrollment in their Q fever IND Immunization Program. For at-risk laboratory workers to participate in this program, fees are applicable. Individuals with valvular heart disease should not work with *C. burnetii*. (See Section VII.)

Select Agent *C. burnetii* is a select agent requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Rickettsia prowazekii; Rickettsia typhi (R. mooseri); Orientia (Rickettsia) tsutsugamushi and Spotted Fever Group agents of human disease; Rickettsia rickettsii, Rickettsia conorii, Rickettsia akari, Rickettsia australis, Rickettsia siberica, and Rickettsia japonicum

Rickettsia prowazekii, Rickettsia typhi (R. mooseri), Orientia (Rickettsia) tsutsugamushi and the Spotted Fever Group agents of human disease (Rickettsia rickettsii, Rickettsia conorii, Rickettsia akari, Rickettsia australis, Rickettsia siberica, and

Rickettsia japonicum) are the etiologic agents of epidemic typhus, endemic (murine) typhus), scrub typhus, Rocky Mountain spotted fever, Mediterranean spotted fever, rickettsialpox, Queensland tick typhus, and North Asian spotted fever, respectively.

Rickettsia spp. are bacterial obligate intracellular pathogens that are transmitted by arthropod vectors and replicate within the cytoplasm of eukaryotic host cells. Two groups are recognized within the genus, the typhus group and the spotted fever group. The more distantly related scrub typhus group is now considered a distinct genus, *Orientia*. Rickettsiae are primarily associated with arthropod vectors in which they may exist as endosymbionts that infect mammals, including humans, through the bite of infected ticks, lice, or fleas.¹⁵

Occupational Infections

Pike reported 57 cases of laboratory-associated typhus (type not specified), 56 cases of epidemic typhus with three deaths, and 68 cases of murine typhus.⁸ Three cases of murine typhus have been reported from a research facility.¹⁶ Two were associated with handling of infectious materials on the open bench; the third case resulted from an accidental parenteral inoculation. These three cases represented an attack rate of 20% in personnel working with infectious materials. Rocky Mountain spotted fever is a documented hazard to laboratory personnel. Pike reported 63 laboratory-associated cases, 11 of which were fatal.⁸ Oster reported nine cases occurring over a six-year period in one laboratory. All were believed to have been acquired because of exposure to infectious aerosols.¹⁷

Natural Modes of Infection

The epidemiology of rickettsial infections reflects the prevalence of rickettsiae in the vector population and the interactions of arthropod vectors with humans. Epidemic typhus is unusual among rickettsiae in that humans are considered the primary host. Transmission is by the human body louse; thus, outbreaks are now associated with breakdowns of social conditions. Endemic typhus is maintained in rodents and transmitted to humans by fleas. The various spotted fever group rickettsiae are limited geographically, probably by the distribution of the arthropod vector, although specific spotted fever group rickettsiae are found on all continents.¹⁵

Laboratory Safety and Containment Recommendations

The necessity of using embryonated eggs or cell culture techniques for the propagation of *Rickettsia* spp. incorporates extensive purification procedures. Accidental parenteral inoculation and exposure to infectious aerosols are the most likely sources of LAI.¹⁸ Aerosol transmission of *R. rickettsii* has been experimentally documented in nonhuman primates.¹⁹ Five cases of rickettsialpox recorded by Pike were associated with exposure to bites of infected mites.⁸ Naturally and experimentally infected mammals, their ectoparasites, and their infected tissues are potential sources of human infection. The organisms are relatively unstable under ambient environmental conditions.

BSL-2 practices, containment equipment, and facilities are recommended for nonpropagative laboratory procedures, including serological and fluorescent antibody procedures, and for the staining of impression smears. BSL-3 practices, containment equipment, and facilities are recommended for all other manipulations of known or potentially infectious materials, including necropsy of experimentally infected animals and trituration of their tissues, and inoculation, incubation, and harvesting of embryonated eggs or cell cultures. ABSL-2 practices, containment equipment, and facilities are recommended for the holding of experimentally infected mammals other than arthropods. BSL-3 practices, containment equipment, and facilities are recommended for animal studies with arthropods naturally or experimentally infected with rickettsial agents of human disease. (See Appendix E.)

Several species, including *R. montana*, *R. rhipicephali*, *R. belli*, and *R. canada*, are not known to cause human disease and may be handled under BSL-2 conditions. New species are being described frequently and should be evaluated for appropriate containment on a case-by-case basis. Because of the proven value of antibiotic therapy in the early stages of rickettsial infection, it is essential that laboratories have an effective system for reporting febrile illnesses in laboratory personnel, medical evaluation of potential cases and, when indicated, institution of appropriate antibiotic therapy.

Special Issues

Medical Response Under natural circumstances, the severity of disease caused by rickettsial agents varies considerably. In the laboratory, very large inocula are possible, which might produce unusual and perhaps very serious responses. Surveillance of personnel for laboratory-associated infections with rickettsial agents can dramatically reduce the risk of serious consequences of disease. Experience indicates that infections adequately treated with specific anti-rickettsial chemotherapy on the first day of disease do not generally present serious problems. However, delay in instituting appropriate chemotherapy may result in debilitating or severe acute disease ranging from increased periods of convalescence in typhus and scrub typhus to death in *R. rickettsii* infections. The key to reducing the severity of disease from laboratory-associated infections is a reliable medical response which includes: 1) round-the-clock availability of an experienced medical officer; 2) indoctrination of all personnel on the potential hazards of working with rickettsial agents and advantages of early therapy; 3) a reporting system for all recognized overt exposures and accidents; 4) the reporting of all febrile illnesses, especially those associated with headache, malaise, and prostration when no other certain cause exists; and 5) an open and non-punitive atmosphere that encourages reporting of any febrile illness.

Select Agent *R. prowazekii* and *R. rickettsii* are select agents requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS.

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Section VIII-E: Viral Agents

Hantaviruses

Hantaviruses are negative sense RNA viruses belonging to the genus *Hantavirus* within the family *Bunyaviridae*. The natural hosts of hantaviruses are rodent species and they occur worldwide. Hantavirus pulmonary syndrome (HPS) is a severe disease caused by hantaviruses such as Sin Nombre virus or Andes virus whose hosts are rodents in the subfamily *Sigmodontinae*. This subfamily only occurs in the New World, so HPS is not seen outside North and South America. Hantaviruses in Europe and Asia frequently cause kidney disease, called nephropathica epidemica in Europe, and hemorrhagic fever with renal syndrome (HFRS) in Asia.

Occupational Infections

Documented laboratory-acquired infections have occurred in individuals working with hantaviruses.¹⁻⁴ Extreme caution must be used in performing any laboratory operation that may create aerosols (centrifugation, vortex-mixing, etc.). Operations involving rats, voles, and other laboratory rodents, should be conducted with special caution because of the extreme hazard of aerosol infection, especially from infected rodent urine.

Natural Modes of Infection

HPS is a severe, often fatal disease that is caused by Sin Nombre and Andes or related viruses.^{5,6} Most cases of human illness have resulted from exposures to naturally infected wild rodents or to their excreta. Person-to-person transmission does not occur, with the exception of a few rare instances documented for Andes virus.⁷ Arthropod vectors are not known to transmit hantaviruses.

Laboratory Safety and Containment Recommendations

Laboratory transmission of hantaviruses from rodents to humans via the aerosol route is well documented.^{4,7} Exposures to rodent excreta, especially aerosolized infectious urine, fresh necropsy material, and animal bedding are presumed to be associated with risk. Other potential routes of laboratory infection include ingestion, contact of infectious materials with mucous membranes or broken skin and, in particular, animal bites. Viral RNA has been detected in necropsy specimens and in patient blood and plasma obtained early in the course of HPS;^{8,9} however, the infectivity of blood or tissues is unknown.

BSL-2 practices, containment equipment, and facilities are recommended for laboratory handling of sera from persons potentially infected with hantaviruses. The use of a certified BSC is recommended for all handling of human body fluids when potential exists for splatter or aerosol.

Potentially infected tissue samples should be handled in BSL-2 facilities following BSL-3 practices and procedures. Cell-culture virus propagation and purification should be carried out in a BSL-3 facility using BSL-3 practices, containment equipment and procedures.

Experimentally infected rodent species known not to excrete the virus can be housed in ABSL-2 facilities using ABSL-2 practices and procedures. Primary physical containment devices including BSCs should be used whenever procedures with potential for generating aerosols are conducted. Serum or tissue samples from potentially infected rodents should be handled at BSL-2 using BSL-3 practices, containment equipment and procedures. All work involving inoculation of virus-containing samples into rodent species permissive for chronic infection should be conducted at ABSL-4.

Special Issues

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS.

Hendra Virus (formerly known as Equine Morbillivirus) and Nipah Virus

Hendra virus and *Nipah* virus are members of a newly recognized genus called *Henipavirus*, within the family *Paramyxoviridae*. Outbreaks of a previously unrecognized paramyxovirus, at first called equine morbillivirus, later named *Hendra* virus, occurred in horses in Australia in 1994 and 1995. During 1998-1999, an outbreak of illness caused by a similar but distinct virus, now known as *Nipah* virus, occurred in Malaysia and Singapore. Human illness, characterized by fever, severe headache, myalgia and signs of encephalitis occurred in individuals in close contact with pigs (i.e., pig farmers and abattoir workers).¹⁰⁻¹⁴ A few patients developed a respiratory disease. Approximately 40% of patients with encephalitis died. Recently, cases of *Nipah* virus infection were described in Bangladesh, apparently the result of close contact with infected fruit bats without an intermediate (e.g., pig) host.

Occupational Infections

No laboratory-acquired infections are known to have occurred because of *Hendra* or *Nipah* virus exposure; however, three people in close contact with ill horses developed encephalitis or respiratory disease and two died.¹⁵⁻²⁰

Natural Modes of Infection

The natural reservoir hosts for the *Hendra* and *Nipah* viruses appear to be fruit bats of the genus *Pteropus*.²¹⁻²³ Studies suggest that a locally occurring member

of the genus, *Pteropus giganteus*, is the reservoir for the virus in Bangladesh.²⁴ Individuals who had regular contact with bats had no evidence of infection (antibody) in one study in Australia.²⁵

Laboratory Safety and Containment Recommendations

The exact mode of transmission of these viruses has not been established. Most clinical cases to date have been associated with close contact with horses, their blood or body fluids (Australia) or pigs (Malaysia/Singapore) but presumed direct transmission from *Pteropus* bats has been recorded in Bangladesh. Hendra and Nipah viruses have been isolated from tissues of infected animals. In the outbreaks in Malaysia and Singapore, viral antigen was found in central nervous system, kidney and lung tissues of fatal human cases²⁶ and virus was present in secretions of patients, albeit at low levels.²⁷ Active surveillance for infection of healthcare workers in Malaysia has not detected evidence of occupationally acquired infections in this setting.²⁸

Because of the unknown risks to laboratory workers and the potential impact on indigenous livestock should the virus escape a diagnostic or research laboratory, health officials and laboratory managers should evaluate the need to work with the virus and the containment capability of the facility before undertaking any work with Hendra, Nipah or suspected related viruses. BSL-4 is required for all work with these viruses. Once a diagnosis of Nipah or Hendra virus is suspected, all diagnostic specimens also must be handled at BSL-4. ABSL-4 is required for any work with infected animals.

Special Issues

Select Agent Hendra and Nipah virus are select agents requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Hepatitis A Virus, Hepatitis E Virus

Hepatitis A virus is a positive single-stranded RNA virus, the type species of the Hepatovirus genus in the family Picornaviridae. Hepatitis E virus is a positive single-stranded RNA virus, the type species of the genus Hepevirus, a floating genus not assigned to any family.

Occupational Infections

Laboratory-associated infections with hepatitis A or E viruses do not appear to be an important occupational risk among laboratory personnel. However, hepatitis A is a documented hazard in animal handlers and others working with naturally or experimentally infected chimpanzees and other nonhuman primates.²⁹ Workers handling other recently captured, susceptible primates (owl monkeys, marmosets) also may be at risk for hepatitis A infection. Hepatitis E virus appears to be less of a risk to personnel than hepatitis A virus, except during pregnancy, when infection can result in severe or fatal disease.

Natural Modes of Infection

Most infections with hepatitis A are foodborne and occasionally water-borne. The virus is present in feces during the prodromal phase of the disease and usually disappears once jaundice occurs. Hepatitis E virus causes acute enterically-transmitted cases of hepatitis, mostly waterborne. In Asia, epidemics involving thousands of cases have occurred.

Laboratory Safety and Containment Recommendations

The agents may be present in feces and blood of infected humans and nonhuman primates. Feces, stool suspensions, and other contaminated materials are the primary hazards to laboratory personnel. Care should be taken to avoid puncture wounds when handling contaminated blood from humans or nonhuman primates. There is no evidence that aerosol exposure results in infection.

BSL-2 practices, containment equipment, and facilities are recommended for the manipulation of hepatitis A and E virus, infected feces, blood or other tissues. ABSL-2 practices and facilities are recommended for activities using naturally or experimentally-infected nonhuman primates or other animal models that may shed the virus.

Special Issues

Vaccines A licensed inactivated vaccine against hepatitis A is available. Vaccines against hepatitis E are not currently available.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Hepatitis B Virus, Hepatitis C Virus (formerly known as nonA nonB Virus), Hepatitis D Virus

Hepatitis B virus (HBV) is the type species of the *Orthohepadnavirus* genus in the family *Hepadnaviridae*. Hepatitis C virus (HCV) is the type species of the *Hepacivirus* genus in the family *Flaviviridae*. Hepatitis D virus (HDV) is the only member of the genus *Deltavirus*.

These viruses are naturally acquired from a carrier during blood transfusion, vaccination, tattooing, or body piercing with inadequately sterilized instruments. Non-parenteral routes, such as domestic contact and unprotected (heterosexual and homosexual) intercourse, are also major modes of transmission.

Individuals who are infected with the HBV are at risk of infection with HDV, a defective RNA virus that requires the presence of HBV virus for replication. Infection with HDV usually exacerbates the symptoms caused by HBV infection.

Occupational Infections

Hepatitis B has been one of the most frequently occurring laboratory-associated infections, and laboratory workers are recognized as a high-risk group for acquiring such infections.³⁰

Hepatitis C virus infection can occur in the laboratory situation as well.³¹ The prevalence of antibody to hepatitis C (anti-HCV) is slightly higher in medical care workers than in the general population. Epidemiologic evidence indicates that HCV is spread predominantly by the parenteral route.³²

Laboratory Safety and Containment Recommendations

HBV may be present in blood and blood products of human origin, in urine, semen, CSF and saliva. Parenteral inoculation, droplet exposure of mucous membranes, and contact exposure of broken skin are the primary laboratory hazards.³³ The virus may be stable in dried blood or blood components for several days. Attenuated or avirulent strains have not been identified.

HCV has been detected primarily in blood and serum, less frequently in saliva and rarely or not at all in urine or semen. It appears to be relatively unstable to storage at room temperature and repeated freezing and thawing.

BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious body fluids and tissues. Additional primary containment and personnel precautions, such as those described for BSL-3, may be indicated for activities with potential for droplet or aerosol production and for activities involving production quantities or concentrations of infectious materials. ABSL-2 practices, containment equipment and facilities are recommended for activities utilizing naturally or experimentally infected chimpanzees or other NHP. Gloves should be worn when working with

infected animals and when there is the likelihood of skin contact with infectious materials. In addition to these recommended precautions, persons working with HBV, HCV, or other bloodborne pathogens should consult the OSHA Bloodborne Pathogen Standard.³⁴ Questions related to interpretation of this Standard should be directed to federal, regional or state OSHA offices.

Special Issues

Vaccines Licensed recombinant vaccines against hepatitis B are available and are highly recommended for and offered to laboratory personnel.³⁵ Vaccines against hepatitis C and D are not yet available for use in humans, but vaccination against HBV will also prevent HDV infection.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS.

Herpesvirus Simiae (Cercopithecine Herpesvirus I, Herpes B Virus)

B virus is a member of the *alphaherpesvirus* genus (simplexvirus) in the family *Herpesviridae*. It occurs naturally in macaque monkeys, of which there are nine distinct species. Macaques may have primary, recurrent, or latent infections often with no apparent symptoms or lesions. B virus is the only member of the family of simplex herpesviruses that can cause zoonotic infections. Human infections have been identified in at least 50 instances, with approximately 80% mortality when untreated. There remains an approximate 20% mortality in the absence of timely treatment with antiviral agents.³⁶ There have been no reported cases where prompt first aid with wound or exposure site cleansing was performed, and no cases where cleaning and post exposure prophylaxis were done. Cases prior to 1970 were not treated with antiviral agents because none were available. Morbidity and mortality associated with zoonotic infection results from invasion of the central nervous system, resulting in ascending paralysis ultimately with loss of ability to sustain respiration in the absence of mechanical ventilation. From 1987-2004, five additional fatal infections bring the number of lethal infections to 29 since the discovery of B virus in 1933.

Occupational Infections

B virus is a hazard in facilities where macaque monkeys are present. Mucosal secretions (saliva, genital secretions, and conjunctival secretions) are the primary body fluids associated with risk of B virus transmission. However, it is possible for other materials to become contaminated. For instance, a research assistant at the Yerkes Primate Center who died following mucosal splash without injury in 1997 was splashed with something in the eye while transporting a caged macaque. In part on this basis, the eye splash was considered low risk. However,

feces, urine or other fluids may be contaminated with virus shed from mucosal fluids. Zoonoses have been reported following virus transmission through a bite, scratch, or splash accident. Cases of B virus have also been reported after exposure to monkey cell cultures and to central nervous system tissue. There is often no apparent evidence of B virus infection in the animals or their cells and tissues, making it imperative that all suspect exposures be treated according to recommended standards.³⁶ The risks associated with this hazard are, however, readily reduced by practicing barrier precautions and by rapid and thorough cleansing immediately following a possible site contamination. Precautions should be followed when work requires the use of any macaque species, even antibody negative animals. In most documented cases of B virus zoonosis, virus was not recovered from potential sources except in four cases, making speculations that some macaque species may be safer than others unfounded. The loss of five lives in the past two decades underscores that B virus infections have a low probability of occurrence, but when they do occur it is with high consequences.

Specific, regular training in risk assessments for B virus hazards including understanding the modes of exposure and transmission should be provided to individuals encountering B virus hazards. This training should include proper use of personal protective equipment, which is essential to prevention. Immediate and thorough cleansing following bites, scratches, splashes, or contact with potential fomites in high-risk areas appears to be helpful in prevention of B virus infections.³⁷ First aid and emergency medical assistance procedures are most effective when institutions set the standard to be practiced by all individuals encountering B virus hazards.

Natural Modes of Infection

B virus occurs as a natural infection of Asiatic macaque monkeys, and some 10% of newly caught rhesus monkeys have antibodies against the virus, which is frequently present in kidney cell cultures of this animal.

Reservoir species include *Macaca mulatta*, *M. fascicularis*, *M. fusata*, *M. arctoides*, *M. cyclopsis* and *M. radiata*. In these species the virus causes vesicular lesions on the tongue and lips, and sometimes of the skin. B virus is not present in blood or serum in infected macaques. Transmission of B virus appears to increase when macaques reach sexual maturity.

Laboratory Safety and Containment Recommendations

The National Academies Press has recently published ILAR's guidelines for working with nonhuman primates.³⁸ Additional resources are provided in the references following this agent summary statement. Asymptomatic B virus shedding accounts for most transmission among monkeys and human workers, but those working in the laboratory with potentially infected cells or tissues from macaques are also at risk. Exposure of mucous membranes or through skin

breaks provides this agent access to a new host, whether the virus is being shed from a macaque or human, or present in or on contaminated cells, tissues, or surfaces.³⁶ B virus is not generally found in serum or blood, but these products obtained through venipuncture should be handled carefully because contamination of needles via skin can occur. When working with macaques directly, virus can be transmitted through bites, scratches, or splashes only when the animal is shedding virus from mucosal sites. Fomites, or contaminated surfaces (e.g., cages, surgical equipment, tables), should always be considered sources of B virus unless verified as decontaminated or sterilized. Zoonotically infected humans should be cautioned about autoinoculation of other susceptible sites when shedding virus during acute infection.

BSL-2 practices and facilities are suitable for all activities involving the use or manipulation of tissues, cells, blood, or serum from macaques with appropriate personal protective equipment. BSL-3 practices are recommended for handling materials from which B virus is being cultured using appropriate personal protective equipment, and BSL-4 facilities are recommended for propagation of virus obtained from diagnostic samples or stocks. Experimental infections of macaques as well as small animal models with B virus are recommended to be restricted to BSL-4 containment.

All macaques regardless of their origin should be considered potentially infected. Animals with no detectable antibody are not necessarily B virus-free. Macaques should be handled with strict barrier precaution protocols and injuries should be tended immediately according to the recommendations of the B Virus Working Group led by NIH and CDC.³⁶

Barrier precautions and appropriate first aid are the keys to prevention of severe morbidity and mortality often associated with B virus zoonoses. These prevention tools were not implemented in each of the five B virus fatalities during the past two decades. Guidelines are available for safely working with macaques and should be consulted.^{36,39} The correct use of gloves, masks, and protective coats, gowns, aprons, or overalls is recommended for all personnel while working with non-human primates, especially macaques and other Old World species, including for all persons entering animal rooms where non-human primates are housed. To minimize the potential for mucous membrane exposure, some form of barrier is required to prevent droplet splashes to eyes, mouth, and nasal passages. Types and use of personal protective equipment (e.g., goggles or glasses with solid side shields and masks, or wrap-around face shields) should be determined with reference to the institutional risk assessment. Specifications of protective equipment must be balanced with the work to be performed so that the barriers selected do not increase work place risk by obscuring vision and contributing to increased risk of bites, needle sticks, scratches, or splashes.

Special Issues

Post-exposure prophylaxis with oral acyclovir or valacyclovir should be considered for significant exposures to B virus. Therapy with intravenous acyclovir and/or ganciclovir in documented B virus infections is also important in reduction of morbidity following B virus zoonotic infection.³⁶ In selected cases, IND permission has been granted for therapy with experimental antiviral drugs. Because of the seriousness of B virus infection, experienced medical and laboratory personnel should be consulted to develop individual case management. Barrier precautions should be observed with confirmed cases. B virus infection, as with all alphaherpesviruses, is lifelong in macaques.⁴⁰ There are no effective vaccines available.

Select Agent B virus is a select agent requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS.

Human Herpes Virus

The herpesviruses are ubiquitous human pathogens and are commonly present in a variety of clinical materials submitted for virus isolation. Thus far, nine herpesviruses have been isolated from humans: herpes simplex virus-1 (HSV-1), HSV-2, human cytomegalovirus (HCMV), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), and human herpesviruses (HHV) 6A, 6B, 7, and 8.⁴¹

HSV infection is characterized by a localized primary lesion. Primary infection with HSV-1 may be mild and unapparent occurring in early childhood. In approximately 10% of infections, overt illness marked by fever and malaise occurs. HSV-1 is a common cause of meningoencephalitis. Genital infections, usually caused by HSV-2, generally occur in adults and are sexually transmissible. Neonatal infections are most frequently caused by HSV-2 but HSV-1 infections are also common. In the neonate, disseminated disease and encephalitis are often fatal. EBV is the cause of infectious mononucleosis. It is also associated with the pathogenesis of several lymphomas and nasopharyngeal cancer.⁴² EBV is serologically distinct from the other herpesviruses; it infects and transforms B-lymphocytes. HCMV infection is common and often undiagnosed presenting as a nonspecific febrile illness. HCMV causes up to 10% of all cases of mononucleosis in young adults. The most severe form of the disease is seen in infants infected *in utero*. Children surviving infection may evidence mental retardation, microcephaly, motor disabilities and chronic liver disease.⁴² HCMV is one of the most common congenital diseases.

VZV is the causative agent of chickenpox and herpes zoster. Chickenpox usually occurs in childhood and zoster occurs more commonly in adults. HHV-6 is the causative agent of exanthema subitum (roseola), a common childhood exanthem.⁴³ Nonspecific febrile illness and febrile seizures are also clinical manifestations of disease. HHV-6 may reactivate in immunocompetent individuals during pregnancy or during critical illness. Two distinct variants, HHV-6A and HHV-6B, exist, the latter causing roseola. HHV-7 is a constitutive inhabitant of adult human saliva.⁴⁴ Clinical manifestations are less well understood but the virus has also been associated with roseola. HHV-8, also known as Kaposi's sarcoma-associated virus, was first identified by Chang and co-workers in 1994.⁴² HHV-8 is believed to be the causative agent of Kaposi's sarcoma and has been associated with primary effusion lymphoma.⁴⁵ The natural history of HHV-8 has not been completely elucidated. High risk groups for HHV-8 include HIV-infected men who have sex with men and individuals from areas of high endemicity, such as Africa or the Mediterranean.⁴⁵ The prevalence of HHV-8 is also higher among intravenous drug users than in the general population.⁴⁵ At least one report has provided evidence that in African children, HHV-8 infection may be transmitted from mother to child.⁴⁶ While few of the human herpesviruses have been demonstrated to cause laboratory-acquired infections, they are both primary and opportunistic pathogens, especially in immunocompromised hosts. Herpesvirus simiae (B-virus, Monkey B virus) is discussed separately in another agent summary statement in this section.

Occupational Infections

Few of the human herpesviruses have been documented as sources of laboratory acquired infections.

In a limited study, Gartner and co-workers have investigated the HHV-8 immunoglobulin G (IgG) seroprevalence rates for healthcare workers caring for patients with a high risk for HHV-8 infection in a non-endemic area. Healthcare workers in contact with risk group patients were infected more frequently than healthcare workers without contact with risk groups. Workers without contact with risk group patients were infected no more frequently than the control group.⁵³

Although this diverse group of indigenous viral agents has not demonstrated a high potential hazard for laboratory-associated infection, frequent presence in clinical materials and common use in research warrant the application of appropriate laboratory containment and safe practices.

Natural Modes of Infection

Given the wide array of viruses included in this family, the natural modes of infection vary greatly, as does the pathogenesis of the various viruses. Some have wide host ranges, multiply effectively, and rapidly destroy the cells they infect (HSV-1, HSV-2). Others have restricted host ranges or long replicative

cycles (HHV-6).⁴¹ Transmission of human herpesviruses in nature are, in general, associated with close, intimate contact with a person excreting the virus in their saliva, urine, or other bodily fluids.⁴⁷ VZV is transmitted person-to-person through direct contact, through aerosolized vesicular fluids and respiratory secretions, and indirectly transmitted by fomites. Latency is a trait common to most herpesviruses, although the site and duration vary greatly. For example, EBV will persist in an asymptomatic, latent form in the host immune system, primarily in EBV-specific cytotoxic T cells⁴² while latent HSV has been detected only in sensory neurons.^{48,49} HHV-8 has been transmitted through organ transplantation⁵⁰ and blood transfusion;⁵¹ some evidence suggests non-sexual horizontal transmission.⁵²

Laboratory Safety and Containment Recommendations

Clinical materials and isolates of herpesviruses may pose a risk of infection following ingestion, accidental parenteral inoculation, and droplet exposure of the mucous membranes of the eyes, nose, or mouth, or inhalation of concentrated aerosolized materials. HHV-8 may be present in human blood or blood products and tissues or saliva. Aerosol transmission cannot be excluded as a potential route of transmission. Clinical specimens containing the more virulent Herpesvirus simiae (B-virus) may be inadvertently submitted for diagnosis of suspected herpes simplex infection. HCMV may pose a special risk during pregnancy because of potential infection of the fetus. All human herpesviruses pose an increased risk to persons who are immunocompromised.

BSL-2 practices, containment equipment, and facilities are recommended for activities utilizing known or potentially infectious clinical materials or cultures of indigenous viral agents that are associated or identified as a primary pathogen of human disease. Although there is little evidence that infectious aerosols are a significant source of LAI, it is prudent to avoid the generation of aerosols during the handling of clinical materials or isolates, or during the necropsy of animals. Primary containment devices (e.g., BSC) should be utilized to prevent exposure of workers to infectious aerosols. Additional containment and procedures, such as those described for BSL-3, should be considered when producing, purifying, and concentrating human herpesviruses, based on risk assessment.

Containment recommendations for herpesvirus simiae (B-virus, Monkey B virus) are described in the preceding agent summary statement.

Special Issues

Vaccine A live, attenuated vaccine for varicella zoster is licensed and available in the United States. In the event of a laboratory exposure to a non-immune individual, varicella vaccine is likely to prevent or at least modify disease.⁴⁷

Treatment Antiviral medications are available for treatment of several of the herpesviruses.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Influenza

Influenza is an acute viral disease of the respiratory tract. The most common clinical manifestations are fever, headache, malaise, sore throat and cough. GI tract manifestations (nausea, vomiting and diarrhea) are rare but may accompany the respiratory phase in children. The two most important features of influenza are the epidemic nature of illness and the mortality that arises from pulmonary complications of the disease.⁵⁴

The influenza viruses are enveloped RNA viruses belonging to the Orthomyxoviridae. There are three serotypes of influenza viruses, A, B and C. Influenza A is further classified into subtypes by the surface glycoproteins that possess either hemagglutinin (H) or neuraminidase (N) activity. Emergence of completely new subtypes (antigenic shift) occurs at irregular intervals with Type A viruses. New subtypes are responsible for pandemics and can result from reassortment of human and avian influenza virus genes. Antigenic changes within a type or subtype (antigenic drift) of A and B viruses are ongoing processes that are responsible for frequent epidemics and regional outbreaks and make the annual reformulation of influenza vaccine necessary.

Influenza viral infections, with different antigenic subtypes, occur naturally in swine, horses, mink, seals and in many domestic and wild avian species. Interspecies transmission and reassortment of influenza A viruses have been reported to occur among humans and wild and domestic fowl. The human influenza viruses responsible for the 1918, 1957 and 1968 pandemics contained gene segments closely related to those of avian influenza viruses.⁵⁵ Swine influenza has also been isolated in human outbreaks.⁵⁶

Control of influenza is a continuing human and veterinary public health concern.

Occupational Infections

LAI have not been routinely documented in the literature, but informal accounts and published reports indicate that such infections are known to have occurred, particularly when new strains showing antigenic shift or drift are introduced into a laboratory for diagnostic/research purposes.⁵⁶ Occupationally-acquired, nosocomial infections are documented.^{57,58} Laboratory animal-associated infections have not been reported; however, there is possibility of human infection acquired from infected ferrets and vice versa.

Natural Modes of Infection

Airborne spread is the predominant mode of transmission especially in crowded, enclosed spaces. Transmission may also occur through direct contact since influenza viruses may persist for hours on surfaces particularly in the cold and under conditions of low humidity.⁵⁵ The incubation period is from one to three days. Recommendations for treatment and prophylaxis of influenza are available.⁵⁹

Laboratory Safety and Containment Recommendations

The agent may be present in respiratory tissues or secretions of humans and most infected animals and birds. In addition, the agent may be present in the intestines and cloacae of many infected avian species. Influenza viruses may be disseminated in multiple organs in some infected animal species. The primary laboratory hazard is inhalation of virus from aerosols generated by infecting animals or by aspirating, dispensing, mixing, centrifuging or otherwise manipulating virus-infected samples. In addition, laboratory infection can result from direct inoculation of mucus membranes through virus-contaminated gloves following handling of tissues, feces or secretions from infected animals. Genetic manipulation has the potential for altering the host range, pathogenicity, and antigenic composition of influenza viruses. The potential for introducing influenza viruses with novel genetic composition into humans is unknown.

BSL-2 facilities, practices and procedures are recommended for diagnostic, research and production activities utilizing contemporary, circulating human influenza strains (e.g., H1/H3/B) and low pathogenicity avian influenza (LPAI) strains (e.g., H1-4, H6, H8-16), and equine and swine influenza viruses. ABSL-2 is appropriate for work with these viruses in animal models. All avian and swine influenza viruses require an APHIS permit. Based on economic ramifications and source of the virus, LPAI H5 and H7 and swine influenza viruses may have additional APHIS permit-driven containment requirements and personnel practices and/or restrictions.

Non-Contemporary Human Influenza (H2N2) Strains

Non-contemporary, wild-type human influenza (H2N2) strains should be handled with increased caution. Important considerations in working with these strains are the number of years since an antigenically related virus last circulated and the potential for presence of a susceptible population. BSL-3 and ABSL-3 practices, procedures and facilities are recommended with rigorous adherence to additional respiratory protection and clothing change protocols. Negative pressure, HEPA-filtered respirators or positive air-purifying respirators (PAPRs) are recommended for use. Cold-adapted, live attenuated H2N2 vaccine strains may continue to be worked with at BSL-2.

1918 Influenza Strain

Any research involving reverse genetics of the 1918 influenza strain should proceed with *extreme* caution. The risk to laboratory workers is unknown, but the pandemic potential is thought to be significant. Until further risk assessment data are available, the following practices and conditions are recommended for manipulation of reconstructed 1918 influenza viruses and laboratory animals infected with the viruses. These practices and procedures are considered minimum standards for work with the fully reconstructed virus.

- BSL-3 and ABSL-3 practices, procedures and facilities.
- Large laboratory animals such as NHP should be housed in primary barrier systems in ABSL-3 facilities.
- Rigorous adherence to additional respiratory protection and clothing change protocols.
- Use of negative pressure, HEPA-filtered respirators or PAPRs.
- Use of HEPA filtration for treatment of exhaust air.
- Amendment of personnel practices to include personal showers prior to exiting the laboratory.

Highly Pathogenic Avian Influenza (HPAI)

Manipulating HPAI viruses in biomedical research laboratories requires similar caution because some strains may pose increased risk to laboratory workers and have significant agricultural and economic implications. BSL-3 and ABSL-3 practices, procedures and facilities are recommended along with clothing change and personal showering protocols. Loose-housed animals infected with HPAI strains must be contained within BSL-3-Ag facilities. (See Appendix D.) Negative pressure, HEPA-filtered respirators or positive air-purifying respirators are recommended for HPAI viruses with potential to infect humans. The HPAI are agricultural select agents requiring registration of personnel and facilities with the lead agency for the institution (CDC or USDA-APHIS). An APHIS permit is also required. Additional containment requirements and personnel practices and/or restrictions may be added as conditions of the permit.

Other Influenza Recombinant or Reassortant Viruses

When considering the biocontainment level and attendant practices and procedures for work with other influenza recombinant or reassortant viruses, the local IBC should consider but not limit consideration to the following in the conduct of protocol-driven risk assessment.

- The gene constellation used.

- Clear evidence of reduced virus replication in the respiratory tract of appropriate animal models, compared with the level of replication of the wild-type parent virus from which it was derived.
- Evidence of clonal purity and phenotypic stability.
- The number of years since a virus that was antigenically related to the donor of the hemagglutinin and neuraminidase genes last circulated.

If adequate risk assessment data are not available, a more cautious approach utilizing elevated biocontainment levels and practices is warranted. There may be specific requirements regarding the setting of containment levels if your institution is subject to the *NIH Guidelines*.

Special Issues

Occupational Health Considerations Institutions performing work with HPAI and avian viruses that have infected humans; non-contemporary wild-type human influenza strains, including recombinants and reassortants; and viruses created by reverse genetics of the 1918 pandemic strain should develop and implement a specific medical surveillance and response plan. At the minimum these plans should: 1) require storage of baseline serum samples from individuals working with these influenza strains; 2) strongly recommend annual vaccination with the currently licensed influenza vaccine for such individuals; 3) provide employee counseling regarding disease symptoms including fever, conjunctivitis and respiratory symptoms; 4) establish a protocol for monitoring personnel for these symptoms; and 5) establish a clear medical protocol for responding to suspected laboratory-acquired infections. Antiviral drugs (e.g., oseltamivir, amantadine, rimantadine, zanamivir) should be available for treatment and prophylaxis, as necessary.⁵⁹ It is recommended that the sensitivities of the virus being studied to the antivirals be ascertained. All personnel should be enrolled in an appropriately constituted respiratory protection program.

Influenza viruses may require USDA and/or USPHS import permits depending on the host range and pathogenicity of the virus in question.

Select Agent Strains of HPAI and 1918 influenza virus are select agents requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Lymphocytic Choriomeningitis Virus

Lymphocytic choriomeningitis (LCM) is a rodent-borne viral infectious disease that presents as aseptic meningitis, encephalitis, or meningoencephalitis. The causative agent is the LCM virus (LCMV) that was initially isolated in 1933. The virus is the prototypical member of the family *Arenaviridae*.

Occupational Infections

LAI with LCM virus are well documented. Most infections occur when chronic viral infection exists in laboratory rodents, especially mice, hamsters and guinea pigs.⁶⁰⁻⁶² Nude and severe combined immune deficient (SCID) mice may pose a special risk of harboring silent chronic infections. Inadvertently infected cell cultures also represent a potential source of infection and dissemination of the agent.

Natural Modes of Infection

LCM and milder LCMV infections have been reported in Europe, the Americas, Australia, and Japan, and may occur wherever infected rodent hosts of the virus are found. Several serologic studies conducted in urban areas have shown that the prevalence of LCMV infection among humans ranges from 2% to 10%. Seroprevalence of 37.5% has been reported in humans in the Slovak Republic.⁶³

The common house mouse, *Mus musculus*, naturally spreads LCMV. Once infected, these mice can become chronically infected as demonstrated by the presence of virus in blood and/or by persistently shedding virus in urine. Infections have also occurred in NHP in zoos, including macaques and marmosets. (*Callitrichid* hepatitis virus is a LCMV.)

Humans become infected by inhaling infectious aerosolized particles of rodent urine, feces, or saliva; by ingesting food contaminated with virus; by contamination of mucous membranes with infected body fluids; or by directly exposing cuts or other open wounds to virus-infected blood. Four recipients of organs from a donor who had unrecognized disseminated LCMV infection sustained severe disease and three succumbed. The source of donor infection was traced to a pet hamster that was not overtly ill.⁶⁴

Laboratory Safety and Containment Recommendations

The agent may be present in blood, CSF, urine, secretions of the nasopharynx, feces and tissues of infected animal hosts and humans. Parenteral inoculation, inhalation, contamination of mucous membranes or broken skin with infectious tissues or fluids from infected animals are common hazards. Aerosol transmission is well documented.⁶⁰

Of special note, tumors may acquire LCMV as an adventitious virus without obvious effects on the tumor. Virus may survive freezing and storage in liquid nitrogen for long periods. When infected tumor cells are transplanted,

subsequent infection of the host and virus excretion may ensue. Pregnant women infected with LCMV have transmitted the virus to their fetuses with death or serious central nervous system malformation as a consequence.⁶⁵

BSL-2 practices, containment equipment, and facilities are suitable for activities utilizing known or potentially infectious body fluids, and for cell culture passage of laboratory-adapted strains. BSL-3 is required for activities with high potential for aerosol production, work with production quantities or high concentrations of infectious materials, and for manipulation of infected transplantable tumors, field isolates and clinical materials from human cases. Strains of LCMV that are shown to be lethal in non-human primates should be handled at BSL-3. ABSL-2 practices, containment equipment, and facilities are suitable for studies in adult mice with mouse brain-passaged strains requiring BSL-2 containment. Work with infected hamsters also should be done at ABSL-3.

Special Issues

Vaccines Vaccines are not available for use in humans.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Poliovirus

Poliovirus is the type species of the *Enterovirus* genus in the family *Picornaviridae*. Enteroviruses are transient inhabitants of the gastrointestinal tract, and are stable at acid pH. Picornaviruses are small, ether-insensitive viruses with an RNA genome.

There are three poliovirus serotypes (P1, P2, and P3). Immunity to one serotype does not produce significant immunity to the other serotypes.

Occupational Infections

Laboratory-associated poliomyelitis is uncommon. Twelve cases, including two deaths, were reported between 1941 and 1976.^{62,66} No laboratory-associated poliomyelitis has been reported for nearly 30 years. Both inactivated poliovirus vaccine (IPV) and oral poliovirus vaccine (OPV) are highly effective in preventing disease, but neither vaccine provides complete protection against infection. Poliovirus infections among immunized laboratory workers are uncommon but remain undetermined in the absence of laboratory confirmation. An immunized laboratory worker may unknowingly be a source of poliovirus transmission to unvaccinated persons in the community.⁶⁷

Natural Modes of Infection

At one time poliovirus infection occurred throughout the world. Transmission of wild poliovirus ceased in the United States in 1979, or possibly earlier. A polio eradication program conducted by the Pan American Health Organization led to elimination of polio from the Western Hemisphere in 1991. The Global Polio Eradication Program has dramatically reduced poliovirus transmission throughout the world.

Humans are the only known reservoir of poliovirus, which is transmitted most frequently by persons with unapparent infections. Person-to-person spread of poliovirus via the fecal-oral route is the most important route of transmission, although the oral-oral route may account for some cases.

Laboratory Safety and Containment Recommendations

The agent is present in the feces and in throat secretions of infected persons and in lymph nodes, brain tissue, and spinal cord tissue in fatal cases. For non-immunized persons in the laboratory, ingestion or parenteral inoculation are the primary routes of infection. For immunized persons, the primary risks are the same, except for parenteral inoculation, which likely presents a lower risk. The importance of aerosol exposure is unknown. Laboratory animal-associated infections have not been reported, but infected nonhuman primates should be considered to present a risk.

BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing wild poliovirus infectious culture fluids, environmental samples, and clinical materials. In addition, potentially infectious materials collected for any purpose should be handled at BSL-2. Laboratory personnel working with such materials must have documented polio vaccination. Persons who have had a primary series of OPV or IPV and who are at an increased risk can receive another dose of IPV, but available data do not indicate the need for more than a single lifetime IPV booster dose for adults.⁶⁸ ABSL-2 practices, containment equipment, and facilities are recommended for studies of virulent viruses in animals. Laboratories should use authentic Sabin OPV attenuated strains unless there are strong scientific reasons for working with wild polioviruses.

In anticipation of polio eradication, the WHO recommends destruction of all poliovirus stocks and potential infectious materials if there is no longer a programmatic or research need for such materials.⁶⁹ Institutions/laboratories in the United States that currently retain wild poliovirus infectious or potential infectious material should be on the United States National Inventory maintained by CDC. When one year has elapsed after detection of the last wild poliovirus worldwide, CDC will inform relevant institutions/laboratories about additional containment procedures. Safety recommendations are subject to change based on international polio eradication activities.

Special Issues

When OPV immunization stops, global control and biosafety requirements for wild as well as attenuated (Sabin) poliovirus materials are expected to become more stringent, consistent with the increased consequences of inadvertent transmission to a growing susceptible community.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Poxviruses

Four genera of the subfamily *Chordopoxvirinae*, family *Poxviridae*, (*Orthopoxvirus*, *Parapoxvirus*, *Yatapoxvirus*, and *Molluscipoxvirus*) contain species that can cause lesions on human skin or mucous membranes with mild to severe systemic rash illness in laboratorians. Species within the first three genera mostly arise as zoonotic agents.^{70,71} Laboratory-acquired poxvirus infections of most concern are from the orthopoxviruses that infect humans: variola virus (causes smallpox; human-specific), monkeypox virus (causes smallpox-like disease), *cowpox virus* (causes skin pustule, generalized rash), and vaccinia virus (causes skin pustule, systemic illness).⁷⁰⁻⁷⁵

Occupational Infections

Vaccinia virus, the leading agent of laboratory-acquired poxvirus infections, is used to make the current smallpox vaccine and may occur as a rare zoonosis.^{70,71} Laboratory-acquired infections with standard, mutant, or bioengineered forms of vaccinia virus have occurred, even in previously vaccinated laboratorians. In addition, vaccination with live vaccinia virus sometimes has side effects, which range from mild events (e.g., fever, fatigue, swollen lymph nodes) to rare, severe, and at times fatal outcomes (e.g., generalized vaccinia, encephalitis, vaccinia necrosum, eczema vaccinatum, ocular keratitis, corneal infection, fetal infection of pregnancy, and possibly myocardial infarction, myopericarditis, or angina), thus vaccination contraindications should be carefully followed.^{70,73-75}

Natural Modes of Infection

Smallpox has been eradicated from the world since 1980, but monkey pox virus is endemic in rodents in parts of Africa. Importation of African rodents into North America in 2003 resulted in an outbreak of monkeypox in humans.⁷² Molluscum contagiosum, a disease due to *Molluscipoxvirus* infection, results in pearly white lesions that may persist for months in persons immunocompromised for various

reasons, including chronic illness, AIDS, other infections, medications, cancer and cancer therapies, or pregnancy.⁷⁰

Laboratory Safety and Containment Recommendations

Poxviruses are stable in a wide range of environmental temperatures and humidity and may be transmitted by fomites.⁷⁰ Virus may enter the body through mucous membranes, broken skin, or by ingestion, parenteral inoculation or droplet or fine-particle aerosol inhalation. Sources of laboratory-acquired infection include exposure to aerosols, environmental samples, naturally or experimentally infected animals, infectious cultures, or clinical samples, including vesiculopustular rash lesion fluid or crusted scabs, various tissue specimens, excretions and respiratory secretions.

Worldwide, all live variola virus work is to be done only within WHO approved BSL-4/ABSL-4 facilities; one is at the CDC in Atlanta and the other is at the State Research Center of Virology and Biotechnology (VECTOR) in Koltsovo, Russia.⁷⁶

In general, all persons working in or entering laboratory or animal care areas where activities with vaccinia, monkey pox, or cowpox viruses are being conducted should have evidence of satisfactory vaccination. Vaccination is advised every three years for work with monkeypox virus and every 10 years for cowpox and vaccinia viruses (neither vaccination nor vaccinia immunoglobulin protect against poxviruses of other genera).⁷³⁻⁷⁵

ABSL-3 practices, containment equipment, and facilities are recommended for monkeypox work in experimentally or naturally infected animals. BSL-2 facilities with BSL-3 practices are advised if vaccinated personnel perform other work with monkeypox virus. These practices include the use of Class I or II BSCs and barriers, such as safety cups or sealed rotors, for all centrifugations. The *NIH Guidelines* have assessed the risk of manipulating attenuated vaccinia strains (modified virus Ankara [MVA], NYVAC, TROVAC, and ALVAC) in areas where no other human orthopoxviruses are being used and have recommended BSL-1.⁷⁶ However, higher levels of containment are recommended if these strains are used in work areas where other orthopoxviruses are manipulated. Vaccination is not required for individuals working only in laboratories where no other orthopoxviruses or recombinants are handled.⁷⁵ BSL-2 and ABSL-2 plus vaccination are recommended for work with most other poxviruses.

Special Issues

Other Considerations The CDC Web site www.cdc.gov provides information on poxviruses, especially variola and monkeypox viruses, smallpox vaccination, and reporting vaccination adverse events. Clinical and other laboratories using poxviruses and clinicians can phone the CDC Clinician Information Line (877-554-4625) and/or the CDC public information hotline (888-246-2675) concerning variola and other human poxvirus infections, smallpox vaccine, vaccinia

immunoglobulin, poxvirus antiviral drugs, or other treatments or quarantine issues. Contact CDC regarding applications to transfer monkeypox viruses.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Rabies Virus (and related lyssaviruses)

Rabies is an acute, progressive, fatal encephalitis caused by negative-stranded RNA viruses in the genus *Lyssavirus*, family *Rhabdoviridae*.⁷⁷ *Rabies virus* is the representative member (type species) of the genus. Members of the group include Australian bat lyssavirus, Duvenhage virus, European bat lyssavirus¹, European bat lyssavirus², Lagos bat virus, and Mokola virus.

Occupational Infections

Rabies LAI are extremely rare; two have been documented. Both resulted from presumed exposure to high concentrations of infectious aerosols, one generated in a vaccine production facility,⁷⁸ and the other in a research facility.⁷⁹ Naturally or experimentally infected animals, their tissues, and their excretions are a potential source of exposure for laboratory and animal care personnel.

Natural Modes of Infection

The natural hosts of rabies are many bat species and terrestrial carnivores, but most mammals can be infected. The saliva of infected animals is highly infectious, and bites are the usual means of transmission, although infection through superficial skin lesions or mucosa is possible.

Laboratory Safety and Containment Recommendations

When working with infected animals, the highest viral concentrations are present in central nervous system (CNS) tissue, salivary glands, and saliva, but rabies viral antigens may be detected in all innervated tissues. The most likely sources for exposure of laboratory and animal care personnel are accidental parenteral inoculation, cuts, or needle sticks with contaminated laboratory equipment, bites by infected animals, and exposure of mucous membranes or broken skin to infectious tissue or fluids. Infectious aerosols have not been a demonstrated hazard to personnel working with routine clinical materials or conducting diagnostic examinations. Fixed and attenuated strains of virus are presumed to be less hazardous, but the two recorded cases of laboratory-associated rabies resulted from presumed exposure to the fixed Challenge Virus Standard and Street Alabama Dufferin strains, respectively.

BSL-2 and/or ABSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious materials or animals. Pre-exposure rabies vaccination is recommended for all individuals prior to working with lyssaviruses or infected animals, or engaging in diagnostic, production, or research activities with these viruses.⁸⁰ Rabies vaccination also is recommended for all individuals entering or working in the same room where lyssaviruses or infected animals are used. Prompt administration of postexposure booster vaccinations is recommended following recognized exposures in previously vaccinated individuals per current guidelines.⁸¹ For routine diagnostic activities, it is not always feasible to open the skull or remove the brain of an infected animal within a BSC, but it is pertinent to use appropriate methods and personal protection equipment, including dedicated laboratory clothing, heavy protective gloves to avoid cuts or sticks from cutting instruments or bone fragments, and a face shield or PAPR to protect the skin and mucous membranes of the eyes, nose, and mouth from exposure to tissue fragments or infectious droplets.

If a Stryker saw is used to open the skull, avoid contacting brain tissue with the blade of the saw. Additional primary containment and personnel precautions, such as those described for BSL-3, are indicated for activities with a high potential for droplet or aerosol production, and for activities involving large production quantities or high concentrations of infectious materials.

Special Issues

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Retroviruses, including Human and Simian Immunodeficiency Viruses (HIV and SIV)

The family *Retroviridae* is divided into two subfamilies, the *Orthoretrovirinae* with six genera including the Lentivirus genus, which includes HIV-1 and HIV-2. Other important human pathogens are human T-lymphotropic viruses 1 and 2 (HTLV-1 and HTLV-2), members of the Deltaretrovirus genus. The Spumaretrovirinae, with one genus, Spumavirus, contains a variety of NHP viruses (foamy viruses) that can occasionally infect humans in close contact with NHPs.

Occupational Infections

Data on occupational HIV transmission in laboratory workers are collected through two CDC-supported national surveillance systems: surveillance for 1) AIDS, and 2) HIV-infected persons who may have acquired their infection through occupational exposures. For surveillance purposes, laboratory workers are defined as those persons, including students and trainees, who have worked in a clinical or HIV laboratory setting anytime since 1978. Cases reported in these two systems are classified as either documented or possible occupational transmission. Those classified as documented occupational transmission had evidence of HIV seroconversion (a negative HIV-antibody test at the time of the exposure which converted to positive) following a discrete percutaneous or mucocutaneous occupational exposure to blood, body fluids, or other clinical or laboratory specimens. As of June 1998, CDC had reports of 16 laboratory workers (all clinical) in the United States with documented occupational transmission.⁸²

Workers have been reported to develop antibodies to simian immunodeficiency virus (SIV) following exposures. One case was associated with a needle-stick that occurred while the worker was manipulating a blood-contaminated needle after bleeding an SIV-infected macaque monkey.⁸³ Another case involved a laboratory worker who handled macaque SIV-infected blood specimens without gloves. Though no specific incident was recalled, this worker had dermatitis on the forearms and hands while working with the infected blood specimens.⁸⁴ A third worker⁸⁵ was exposed to SIV-infected primate blood through a needle-stick and subsequently developed antibodies to SIV. To date there is no evidence of illness or immunological incompetence in any of these workers.

Natural Modes of Infection

Retroviruses are widely distributed as infectious agents of vertebrates. Within the human population, spread is by close sexual contact or parenteral exposure through blood or blood products.

Laboratory Safety and Containment Recommendations

HIV has been isolated from blood, semen, saliva, tears, urine, CSF, amniotic fluid, breast milk, cervical secretion, and tissues of infected persons and experimentally infected nonhuman primates.⁸⁶

Although the risk of occupationally-acquired HIV is primarily through exposure to infected blood, it is also prudent to wear gloves when manipulating other body fluids such as feces, saliva, urine, tears, sweat, vomitus, and human breast milk. This also reduces the potential for exposure to other microorganisms that may cause other types of infections.

In the laboratory, virus should be presumed to be present in all blood or clinical specimens contaminated with blood, in any unfixed tissue or organ (other

than intact skin) from a human (living or dead), in HIV cultures, in all materials derived from HIV cultures, and in/on all equipment and devices coming into direct contact with any of these materials.

SIV has been isolated from blood, CSF, and a variety of tissues of infected nonhuman primates. Limited data exist on the concentration of virus in semen, saliva, cervical secretions, urine, breast milk, and amniotic fluid. Virus should be presumed to be present in all SIV cultures, in animals experimentally infected or inoculated with SIV, in all materials derived from SIV cultures, and in/on all equipment and devices coming into direct contact with any of these materials.⁸⁷

The skin (especially when scratches, cuts, abrasions, dermatitis, or other lesions are present) and mucous membranes of the eye, nose, and mouth should be considered as potential pathways for entry of these retroviruses during laboratory activities. It is unknown whether infection can occur via the respiratory tract. The need for using sharps in the laboratory should be evaluated. Needles, sharp instruments, broken glass, and other sharp objects must be carefully handled and properly discarded. Care must be taken to avoid spilling and splashing infected cell-culture liquid and other potentially infected materials.⁸⁵

BSL-2 practices, containment equipment, and facilities are recommended for activities involving blood-contaminated clinical specimens, body fluids and tissues. HTLV-1 and HTLV-2 should also be handled at this level. Activities such as producing research-laboratory-scale quantities of HIV or SIV, manipulating concentrated virus preparations, and conducting procedures that may produce droplets or aerosols, are performed in a BSL-2 facility, using BSL-3 practices. Activities involving large-scale volumes or preparation of concentrated HIV or SIV are conducted at BSL-3. ABSL-2 is appropriate for NHP and other animals infected with HIV or SIV. Human serum from any source that is used as a control or reagent in a test procedure should be handled at BSL-2.

In addition to the aforementioned recommendations, persons working with HIV, SIV, or other bloodborne pathogens should consult the OSHA Bloodborne Pathogen Standard.⁸⁸ Questions related to interpretation of this Standard should be directed to federal, regional or state OSHA offices.

Special Issues

It is recommended that all institutions establish written policies regarding the management of laboratory exposure to HIV and SIV, including treatment and prophylaxis protocols. (See Section VII.)

The risk associated with retroviral vector systems can vary significantly, especially lentiviral vectors. Because the risk associated with each gene transfer system can vary, no specific guideline can be offered other than to have all gene transfer protocols reviewed by an IBC.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Severe Acute Respiratory Syndrome (SARS) Coronavirus

SARS is a viral respiratory illness caused by a previously undescribed coronavirus, SARS-associated coronavirus (SARS-CoV) within the family *Coronaviridae*. SARS was retrospectively recognized in China in November 2002. Over the next few months, the illness spread to other south-east.

Asian countries, North America, South America, and Europe following major airline routes. The majority of disease spread occurred in hospitals, among family members and contacts of hospital workers. From November 2002 through July 2003, when the global outbreak was contained, a total of 8,098 probable cases of SARS were reported to the WHO from 29 countries.⁸⁹

In general, SARS patients present with fever (temperature greater than 100.4°F [$>38.0^{\circ}\text{C}$]), malaise and myalgias quickly followed by respiratory symptoms including shortness of breath and cough. Ten to 20 percent of patients may have diarrhea. Review of probable cases indicates that the shortness of breath sometimes rapidly progresses to respiratory failure requiring ventilation. The case fatality rate is about 11%.

Occupational Infections

Healthcare workers are at increased risk of acquiring SARS from an infected patient especially if involved in pulmonary/respiratory procedures such as endotracheal intubation, aerosolization or nebulization of medications, diagnostic sputum induction, airway suctioning, positive pressure ventilation and high-frequency oscillatory ventilation.

Two confirmed episodes of SARS-CoV transmission to laboratory workers occurred in research laboratories in Singapore and Taiwan.^{89,90} Both occurrences were linked to breaches in laboratory practices. Laboratory-acquired infections in China during 2004 demonstrated secondary and tertiary spread of the disease to close contacts and healthcare providers of one of the employees involved.⁹¹ Although no laboratory-acquired cases have been associated with the routine processing of diagnostic specimens, SARS coronavirus represents an emerging infectious disease for which risk to the medical and laboratory community is not fully understood.

Natural Modes of Infection

The mode of transmission in nature is not well understood. It appears that SARS is transmitted from person-to-person through close contact such as caring for, living with, or having direct contact with respiratory secretions or body fluids of a suspect or probable case.⁹² SARS is thought to be spread primarily through droplets, aerosols and possibly fomites. The natural reservoir for SARS CoV is unknown.

Laboratory Safety and Containment Recommendations

SARS-CoV may be detected in respiratory, blood, or stool specimens. The exact mode of transmission of SARS-CoV laboratory-acquired infection has not been established, but in clinical settings the primary mode of transmission appears through direct or indirect contact of mucous membranes with infectious respiratory droplets.^{93,94}

In clinical laboratories, whole blood, serum, plasma and urine specimens should be handled using Standard Precautions, which includes use of gloves, gown, mask, and eye protection. Any procedure with the potential to generate aerosols (e.g., vortexing or sonication of specimens in an open tube) should be performed in a BSC. Use sealed centrifuge rotors or gasketed safety carriers for centrifugation. Rotors and safety carriers should be loaded and unloaded in a BSC. Procedures conducted outside a BSC must be performed in a manner that minimizes the risk of personnel exposure and environmental release.

The following procedures may be conducted in the BSL-2 setting: pathologic examination and processing of formalin-fixed or otherwise inactivated tissues, molecular analysis of extracted nucleic acid preparations, electron microscopic studies with glutaraldehyde-fixed grids, routine examination of bacterial and fungal cultures, routine staining and microscopic analysis of fixed smears, and final packaging of specimens for transport to diagnostic laboratories for additional testing (specimens should already be in a sealed, decontaminated primary container).

Activities involving manipulation of untreated specimens should be performed in BSL-2 facilities following BSL-3 practices. In the rare event that a procedure or process involving untreated specimens cannot be conducted in a BSC, gloves, gown, eye protection, and respiratory protection (acceptable methods of respiratory protection include: a properly fit-tested, National Institute for Occupational Safety and Health [NIOSH]-approved filter respirator [N-95 or higher level] or a PAPR equipped with HEPA filters) should be used. All personnel who use respiratory protective devices should be enrolled in an appropriately constituted respiratory protection program.

Work surfaces should be decontaminated upon completion of work with appropriate disinfectants. All waste must be decontaminated prior to disposal.

SARS-CoV propagation in cell culture and the initial characterization of viral agents recovered in cultures of SARS specimens must be performed in a BSL-3 facility using BSL-3 practices and procedures. Risk assessment may dictate the additional use of respiratory protection.

Inoculation of animals for potential recovery of SARS-CoV from SARS samples, research studies, and protocols involving animal inoculation for characterization of putative SARS agents must be performed in ABSL-3 facilities using ABSL-3 work practices. Respiratory protection should be used as warranted by risk assessment.

In the event of any break in laboratory procedure or accidents (e.g., accidental spillage of material suspected of containing SARS-CoV), procedures for emergency exposure management and/or environmental decontamination should be immediately implemented and the supervisor should be notified. The worker and the supervisor, in consultation with occupational health or infection control personnel, should evaluate the break in procedure to determine if an exposure occurred (see Special Issues, below).

Special Issues

Occupational Health Considerations Institutions performing work with SARS coronavirus should require storage of a baseline serum sample from individuals who work with the virus or virus-containing specimens. Personnel working with the virus or samples containing or potentially containing the virus should be trained regarding the symptoms of SARS-CoV infection and counseled to report any fever or respiratory symptoms to their supervisor immediately. They should be evaluated for possible exposure and the clinical features and course of their illness should be closely monitored. Institutions performing work with the SARS-CoV or handling specimens likely to contain the agent should develop and implement a specific occupational medical plan with respect to this agent. The plan, at a minimum, should contain procedures for managing:

- identifiable breaks in laboratory procedures;
- exposed workers without symptoms;
- exposed workers who develop symptoms within ten days of an exposure; and
- symptomatic laboratory workers with no recognized exposure.

Further information and guidance regarding the development of a personnel exposure response plan is available from the CDC.⁹⁵ Laboratory workers who are believed to have had a laboratory exposure to SARS-CoV should be evaluated, counseled about the risk of SARS-CoV transmission to others, and monitored for fever or lower respiratory symptoms as well as for any of the following: sore throat, rhinorrhea, chills, rigors, myalgia, headache, and diarrhea.

Local and/or state public health departments should be promptly notified of laboratory exposures and illness in exposed laboratory workers.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS.

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Section VIII-F: Arboviruses and Related Zoonotic Viruses

In 1979, the American Committee on Arthropod-Borne Viruses (ACAV) Subcommittee on Arbovirus Laboratory Safety (SALS) first provided biosafety recommendations for each of the 424 viruses then registered in the International Catalogue of Arboviruses, including Certain Other Viruses of Vertebrates.¹ Working together, SALS, the CDC and the NIH have periodically updated the catalogue by providing recommended biosafety practices and containment for arboviruses registered since 1979. These recommendations are based, in part, on risk assessments derived from information provided by a worldwide survey of laboratories working with arboviruses, new published reports on the viruses, as well as discussions with scientists working with each virus.

Table 6, located at the end of this Section, provides an alphabetical listing of 597 viruses and includes common name, virus family or genus, acronym, BSL recommendation, the basis for the rating, the antigenic group² (if known), HEPA filtration requirements, and regulatory requirements (i.e., import/export permits from either the CDC or the USDA). In addition, many of the organisms are classified as select agents and require special security measures to possess, use, or transport. (See Appendix F.) Table 4 provides a key for the SALS basis for assignment of viruses listed in Table 6.

Agent summary statements have been included for certain arboviruses. They were submitted by a panel of experts for more detailed consideration due to one or more of the following factors:

- at the time of writing this edition, the organism represented an emerging public health threat in the United States;
- the organism presented unique biocontainment challenge(s) that required further detail; and
- the organism presented a significant risk of laboratory-acquired infection.

These recommendations were made in August 2005; requirements for biosafety, shipping, and select agent registration can change. Please be sure to confirm the requirements with the appropriate Federal agency. If the pathogen of interest is one listed in Appendix D, contact the USDA for additional biosafety requirements. USDA guidance may supersede the information found in this Chapter.

Recommendations for the containment of infected arthropod vectors were drafted by a subcommittee of the American Committee on Medical Entomology (ACME), and circulated widely among medical entomology professionals. (See Appendix E.)

Some commonly used vaccine strains for which attenuation has been firmly established are recognized by SALS. These vaccine strains may be handled safely at BSL-2 (Table 5). The agents in Table 4 and 5 may require permits from USDA/DOC/DHHS.

Table 4. Explanation of Symbols Used in Table 6 to Define Basis for Assignment of Viruses to Biosafety Levels

Symbol	Definition
S	Results of SALS survey and information from the Catalog. ¹
IE	Insufficient experience with virus in laboratory facilities with low biocontainment.
A	Additional criteria.
A1	Disease in sheep, cattle or horses.
A2	Fatal human laboratory infection—probably aerosol.
A3	Extensive laboratory experience and mild nature of aerosol laboratory infections justifies BSL-2.
A4	Placed in BSL-4 based on the close antigenic relationship with a known BSL-4 agent plus insufficient experience.
A5	BSL-2 arenaviruses are not known to cause serious acute disease in humans and are not acutely pathogenic for laboratory animals including primates. In view of reported high frequency of laboratory aerosol infection in workers manipulating high concentrations of Pichinde virus, it is strongly recommended that work with high concentrations of BSL-2 arenaviruses be done at BSL-3.
A6	Level assigned to prototype or wild-type virus. A lower level may be recommended for variants with well-defined reduced virulence characteristics.
A7	Placed at this biosafety level based on close antigenic or genetic relationship to other viruses in a group of 3 or more viruses, all of which are classified at this level.
A8	BSL-2 hantaviruses are not known to cause laboratory infections, overt disease in humans, or severe disease in experimental primates. Because of antigenic and biologic relationships to highly pathogenic hantaviruses and the likelihood that experimentally infected rodents may shed large amounts of virus, it is recommended that work with high concentrations or experimentally infected rodents be conducted at BSL-3.

Table 5. Vaccine Strains of BSL-3 and BSL-4 Viruses that May Be Handled as BSL-2

Virus	Vaccine Strain
Chikungunya	181/25
Junin	Candid #1
Rift Valley fever	MP-12
Venezuelan equine encephalomyelitis	TC83 & V3526
Yellow fever	17-D
Japanese encephalitis	14-14-2

Based on the recommendations listed with the tables, the following guidelines should be adhered to where applicable.

Viruses with BSL-2 Containment Recommended

The recommendation for conducting work with the viruses listed in Table 6 at BSL-2 are based on the existence of historical laboratory experience adequate to assess the risks when working with this group of viruses. This indicates a) no overt laboratory-associated infections are reported, b) infections resulted from exposures other than by infectious aerosols, or c) if disease from aerosol exposure is documented, it is uncommon.

Laboratory Safety and Containment Recommendations

Agents listed in this group may be present in blood, CSF, various tissues, and/or infected arthropods, depending on the agent and the stage of infection. The primary laboratory hazards comprise accidental parenteral inoculation, contact of the virus with broken skin or mucous membranes, and bites of infected laboratory rodents or arthropods. Properly maintained BSCs, preferable Class II, or other appropriate personal protective equipment or physical containment devices are used whenever procedures with a potential for creating infectious aerosols or splashes are conducted.

BSL-2 practices, containment equipment, and facilities are recommended for activities with potentially infectious clinical materials and arthropods and for manipulations of infected tissue cultures, embryonate hen’s eggs, and rodents.

Large quantities and/or high concentrations of any virus have the potential to overwhelm both innate immune mechanisms and vaccine-induced immunity. When a BSL-2 virus is being produced in large quantities or in high concentrations, additional risk assessment is required. This might indicate BSL-3 practices, including additional respiratory protection, based on the risk assessment of the proposed experiment.

Viruses with BSL-3 Containment Recommended

The recommendations for viruses listed in Table 6 that require BSL-3 containment are based on multiple criteria. SALS considered the laboratory experience for some viruses to be inadequate to assess risk, regardless of the available information regarding disease severity. In some cases, SALS recorded overt LAI transmitted by the aerosol route in the absence or non-use of protective vaccines, and considered that the natural disease in humans is potentially severe, life threatening, or causes residual damage.¹ Arboviruses also were classified as requiring BSL-3 containment if they caused diseases in domestic animals in countries outside of the United States.

Laboratory Safety and Containment Recommendations

The agents listed in this group may be present in blood, CSF, urine, and exudates, depending on the specific agent and stage of disease. The primary laboratory hazards are exposure to aerosols of infectious solutions and animal bedding, accidental parenteral inoculation, and contact with broken skin. Some of these agents (e.g., VEE virus) may be relatively stable in dried blood or exudates.

BSL-3 practices, containment equipment, and facilities are recommended for activities using potentially infectious clinical materials and infected tissue cultures, animals, or arthropods.

A licensed attenuated live virus is available for immunization against yellow fever. It is recommended for all personnel who work with this agent or with infected animals, and those entering rooms where the agents or infected animals are present.

Junin virus has been reclassified to BSL-3, provided that all at-risk personnel are immunized and the laboratory is equipped with HEPA-filtered exhaust. SALS also has reclassified Central European tick-borne encephalitis (CETBE) viruses to BSL-3, provided all at-risk personnel are immunized. CETBE is not a registered name in *The International Catalogue of Arboviruses* (1985). Until the registration issue is resolved taxonomically, CETBE refers to the following group of very closely related, if not essentially identical, tick-borne flaviviruses isolated from Czechoslovakia, Finland and Russia: Absettarov, Hanzalova, Hypr, and Kumlinge viruses. While there is a vaccine available that confers immunity to the CETBE group of genetically (>98%) homogeneous viruses, the efficacy of this vaccine against Russian spring-summer encephalitis (RSSE) virus infections has not been established. Thus, the CETBE group of viruses has been reclassified as BSL-3 when personnel are immunized with CETBE vaccine, while RSSE remains classified as BSL-4. It should be noted that CETBE viruses are currently listed as select agents and require special security and permitting considerations. (See Appendix F.)

Investigational vaccines for eastern equine encephalomyelitis (EEE) virus, Venezuelan equine encephalitis (VEE), western equine encephalomyelitis (WEE) virus, and Rift Valley fever viruses (RVFV), may be available in limited quantities and administered on-site at the Special Immunization Program of USAMRIID, located at Ft. Detrick, Frederick, MD. Details are available at the end of this section.

The use of investigational vaccines for laboratory personnel should be considered if the vaccine is available. Initial studies have shown the vaccine to be effective in producing an appropriate immunologic response, and the adverse effects of vaccination are within acceptable parameters. The decision to recommend vaccines for laboratory personnel must be carefully considered and based on a risk assessment which includes a review of the characteristics of the agent and the disease, benefits versus the risk of vaccination, the experience of the laboratory personnel, laboratory procedures to be used with the agent, and the contraindications for vaccination including the health status of the employee.

If the investigational vaccine is contraindicated, does not provide acceptable reliability for producing an immune response, or laboratory personnel refuse vaccination, the use of appropriate personal protective equipment may provide an alternative. Respiratory protection, such as use of a PAPR, should be considered in areas using organisms with a well-established risk of aerosol infections in the laboratory, such as VEE viruses.

Any respiratory protection equipment must be provided in accordance with the institution's respiratory protection program. Other degrees of respiratory protection may be warranted based on an assessment of risk as defined in Chapter 2 of this manual. All personnel in a laboratory with the infectious agent must use comparable personal protective equipment that meets or exceeds the requirements, even if they are not working with the organism. Sharps precautions as described under BSL-2 and BSL-3 requirements must be continually and strictly reinforced, regardless of whether investigational vaccines are used.

Non-licensed vaccines are available in limited quantities and administered on-site at the Special Immunization Program of USAMRIID. IND vaccines are administered under a cooperative agreement between the U.S. Army and the individual's requesting organization. Contact the Special Immunization Program by telephone at (301) 619-4653.

Enhanced BSL-3 Containment

Situations may arise for which enhancements to BSL-3 practices and equipment are required; for example, when a BSL-3 laboratory performs diagnostic testing on specimens from patients with hemorrhagic fevers thought to be due to dengue or yellow fever viruses. When the origin of these specimens is Africa, the Middle East, or South America, such specimens might contain etiologic agents, such as arenaviruses, filoviruses, or other viruses that are usually manipulated in a BSL-4

laboratory. Examples of enhancements to BSL-3 laboratories might include: 1) enhanced respiratory protection of personnel against aerosols; 2) HEPA filtration of dedicated exhaust air from the laboratory; and 3) personal body shower. Additional appropriate training for all animal care personnel should be considered.

Viruses with BSL-4 Containment Recommended

The recommendations for viruses assigned to BSL-4 containment are based on documented cases of severe and frequently fatal naturally occurring human infections and aerosol-transmitted laboratory infections. SALS recommends that certain agents with a close antigenic relationship to agents assigned to BSL-4 also be provisionally handled at this level until sufficient laboratory data indicates that work with the agent may be assigned to a lower biosafety level.

Laboratory Safety and Containment Recommendations

The infectious agents may be present in blood, urine, respiratory and throat secretions, semen, and other fluids and tissues from human or animal hosts, and in arthropods, rodents, and NHPs. Respiratory exposure to infectious aerosols, mucous membrane exposure to infectious droplets, and accidental parenteral inoculation are the primary hazards to laboratory or animal care personnel.^{3,4}

BSL-4 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious materials of human, animal, or arthropod origin. Clinical specimens from persons suspected of being infected with one of the agents listed in this summary should be submitted to a laboratory with a BSL-4 maximum containment facility.⁵

Dealing with Unknown Arboviruses

The ACAV has published reports documenting laboratory workers who acquired arbovirus infections during the course of their duties.⁶ In the first such document, it was recognized that these laboratory infections typically occurred by unnatural routes such as percutaneous or aerosol exposure, that “lab adapted” strains were still pathogenic for humans, and that as more laboratories worked with newly identified agents, the frequency of laboratory-acquired infections was increasing. Therefore, to assess the risk of these viruses and provide safety guidelines to those working with them, ACAV appointed SALS to evaluate the hazards of working with arboviruses in the laboratory setting.^{7,8}

The SALS committee made a series of recommendations, published in 1980, describing four levels of laboratory practices and containment guidelines that were progressively more restrictive. These levels were determined after widely-distributed surveys evaluated numerous criteria for each particular virus including: 1) past occurrence of laboratory-acquired infections correlated with facilities and practices used; 2) volume of work performed as a measure of

potential exposure risk; 3) immune status of laboratory personnel; 4) incidence and severity of naturally-acquired infections in adults; and 5) incidence of disease in animals outside the United States (to assess import risk).

While these criteria are still important factors to consider in any risk assessment for manipulating arboviruses in the laboratory, it is important to note that there have been many modifications to personal laboratory practices (e.g., working in BSC while wearing extensive personal protective equipment in contrast to working with viruses on an open bench top) and significant changes in laboratory equipment and facilities (e.g., BSC, PAPR) available since the initial SALS evaluation. Clearly, when dealing with a newly recognized arbovirus, there is insufficient previous experience with it; thus, the virus should be assigned a higher biosafety level. However, with increased ability to safely characterize viruses, the relationship to other disease-causing arboviruses can be established with reduced exposure to the investigators. Therefore, in addition to those established by SALS, additional assessment criteria should be considered.

One criterion for a newly identified arbovirus is a thorough description of how the virus will be handled and investigated. For example, experiments involving pure genetic analysis could be handled differently than those where the virus will be put into animals or arthropods.⁹ Additionally, an individual risk assessment should consider the fact that not all strains of a particular virus exhibit the same degree of pathogenicity or transmissibility. While variable pathogenicity occurs frequently with naturally identified strains, it is of particular note for strains that are modified in the laboratory. It may be tempting to assign biosafety levels to hybrid or chimeric strains based on the parental types but due to possible altered biohazard potential, assignment to a different biosafety level may be justified.¹⁰ A clear description of the strains involved should accompany any risk assessment.

Most of the identified arboviruses have been assigned biosafety levels; however, a number of those that are infrequently studied, newly identified, or have only single isolation events may not have been evaluated by SALS, ACAV, CDC, or the NIH (Table 6). Thorough risk assessment is important for all arboviral research and it is of particular importance for work involving unclassified viruses. A careful assessment by the laboratory director, institutional biosafety officer and safety committee, and as necessary, outside experts is necessary to minimize the risk of human, animal, and environmental exposure while allowing research to progress.

Chimeric Viruses

The ability to construct cDNA clones encoding a complete RNA viral genome has led to the generation of recombinant viruses containing a mixture of genes from two or more different viruses. Chimeric, full-length viruses and truncated replicons have been constructed from numerous alphaviruses and flaviviruses. For example, alphavirus replicons encoding foreign genes have been used

widely as immunogens against bunyavirus, filovirus, arenavirus, and other antigens. These replicons have been safe and usually immunogenic in rodent hosts leading to their development as candidate human vaccines against several virus groups including retroviruses.¹¹⁻¹⁴

Because chimeric viruses contain portions of multiple viruses, the IBC, in conjunction with the biosafety officer and the researchers, must conduct a risk assessment that, in addition to standard criteria, includes specific elements that need to be considered before assigning appropriate biosafety levels and containment practices. These elements include: 1) the ability of the chimeric virus to replicate in cell culture and animal model systems in comparison with its parental strains;¹⁵ 2) altered virulence characteristics or attenuation compared with the parental viruses in animal models;¹⁶ 3) virulence or attenuation patterns by intracranial routes using large doses for agents affecting the CNS;^{17,18} and 4) demonstration of lack of reversion to virulence or parental phenotype.

Many patterns of attenuation have been observed with chimeric flaviviruses and alphaviruses using the criteria described above. Additionally, some of these chimeras are in phase II testing as human vaccines.¹⁹

Chimeric viruses may have some safety features not associated with parental viruses. For example, they are generated from genetically stable cDNA clones without the need for animal or cell culture passage. This minimizes the possibility of mutations that could alter virulence properties. Because some chimeric strains incorporate genomic segments lacking gene regions or genetic elements critical for virulence, there may be limited possibility of laboratory recombination to generate strains exhibiting wild-type virulence.

Ongoing surveillance and laboratory studies suggest that many arboviruses continue to be a risk to human and animal populations. The attenuation of all chimeric strains should be verified using the most rigorous containment requirements of the parental strains. The local IBC should evaluate containment recommendations for each chimeric virus on a case-by-case basis, using virulence data from an appropriate animal model. Additional guidance from the NIH Office of Biotechnology Activities and/or the Recombinant DNA Advisory Committee (RAC) may be necessary.

West Nile Virus (WNV)

WNV has emerged in recent years in temperate regions of Europe and North America, presenting a threat to public and animal health. This virus belongs to the family *Flaviviridae* and the genus *Flavivirus*, Japanese encephalitis virus antigenic complex. The complex currently includes Alfuy, Cacipacore, Japanese encephalitis, Koutango, Kunjin, Murray Valley encephalitis, St. Louis encephalitis,

Rocio, Stratford, Usutu, West Nile, and Yaounde viruses. Flaviviruses share a common size (40-60nm), symmetry (enveloped, icosahedral nucleocapsid), nucleic acid (positive-sense, single stranded RNA approximately 10,000-11,000 bases) and virus morphology. The virus was first isolated from a febrile adult woman in the West Nile District of Uganda in 1937.²⁰ The ecology was characterized in Egypt in the 1950s; equine disease was first noted in Egypt and France in the early 1960s.^{21,22} It first appeared in North America in 1999 as encephalitis reported in humans and horses.²³ The virus has been detected in Africa, Europe, the Middle East, west and central Asia, Oceania (subtype Kunjin virus), and most recently, North America.

Occupational Infections

LAI with WNV have been reported in the literature. SALS reported 15 human infections from laboratory accidents in 1980. One of these infections was attributed to aerosol exposure. Two parenteral inoculations have been reported recently during work with animals.²⁴

Natural Modes of Infections

In the United States, infected mosquitoes, primarily members of the *Culex* genus, transmit WNV. Virus amplification occurs during periods of adult mosquito blood-feeding by continuous transmission between mosquito vectors and bird reservoir hosts. People, horses, and most other mammals are not known to develop infectious viremias very often, and thus are probably “dead-end” or incidental hosts.

Laboratory Safety and Containment Recommendations

WNV may be present in blood, serum, tissues, and CSF of infected humans, birds, mammals, and reptiles. The virus has been found in oral fluids and feces of birds. Parenteral inoculation with contaminated materials poses the greatest hazard; contact exposure of broken skin is a possible risk. Sharps precautions should be strictly adhered to when handling potentially infectious materials. Workers performing necropsies on infected animals may be at higher risk of infection.

BSL-2 practices, containment equipment, and facilities are recommended for activities with human diagnostic specimens, although it is unusual to recover virus from specimens obtained from clinically ill patients. BSL-2 is recommended for processing field collected mosquito pools whereas BSL-3 and ABSL-3 practices, containment equipment, and facilities are recommended for all manipulations of WNV cultures and for experimental animal and vector studies, respectively.

Dissection of field collected dead birds for histopathology and culture is recommended at BSL-3 containment due to the potentially high levels of virus found in such samples. Non-invasive procedures performed on dead birds (such as oropharyngeal or cloacal swabs) can be conducted at BSL-2.

Special Issues

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Eastern Equine Encephalitis (EEE) Virus, Venezuelan Equine Encephalitis (VEE) Virus, and Western Equine Encephalitis (WEE) Virus

VEE, EEE, and WEE viruses are members of the genus *Alphavirus* in the family *Togaviridae*. They are small, enveloped viruses with a genome consisting of a single strand of positive-sense RNA. All three viruses can cause encephalitis often accompanied by long-term neurological sequelae. Incubation period ranges from 1-10 days and the duration of acute illness is typically days to weeks depending upon severity of illness. Although not the natural route of transmission, the viruses are highly infectious by the aerosol route; laboratory acquired infections have been documented.²⁵

Occupational Infections

These alphaviruses, especially VEE virus, are infectious by aerosol in laboratory studies and more than 160 EEE virus, VEE virus, or WEE virus laboratory-acquired infections have been documented. Many infections were due to procedures involving high virus concentrations and aerosol-generating activities such as centrifugation and mouth pipetting. Procedures involving animals (e.g., infection of newly hatched chicks with EEE virus and WEE virus) and mosquitoes also are particularly hazardous.

Natural Modes of Infection

Alphaviruses are zoonoses maintained and amplified in natural transmission cycles involving a variety of mosquito species and either small rodents or birds. Humans and equines are accidental hosts with naturally acquired alphavirus infections resulting from the bites of infected mosquitoes.

EEE virus occurs in focal locations along the eastern seaboard, the Gulf Coast and some inland Midwestern locations of the United States, in Canada, some Caribbean Islands, and Central and South America.²⁶ Small outbreaks of human disease have occurred in the United States, the Dominican Republic, Cuba, and Jamaica. In the United States, equine epizootics are common occurrences during the summer in coastal regions bordering the Atlantic and Gulf of Mexico, in other eastern and Midwestern states, and as far north as Quebec, Ontario, and Alberta in Canada.

In Central and South America, focal outbreaks due to VEE virus occur periodically with rare large regional epizootics involving thousands of equine cases and deaths in predominantly rural settings. These epizootic/epidemic viruses are theorized to emerge periodically from mutations occurring in the continuously circulating enzootic VEE viruses in northern South America. The classical epizootic varieties of the virus are not present in the United States. An enzootic subtype, Everglades virus (VEE antigenic complex subtype II virus), exists naturally in southern Florida, while endemic foci of Bijou Bridge virus (VEE antigenic complex subtype III-B virus), have been described in the western United States.²⁷

The WEE virus is found mainly in western parts of the United States and Canada. Sporadic infections also occur in Central and South America.

Laboratory Safety and Containment Recommendations

Alphaviruses may be present in blood, CSF, other tissues (e.g., brain), or throat washings. The primary laboratory hazards are parenteral inoculation, contact of the virus with broken skin or mucus membranes, bites of infected animals or arthropods, or aerosol inhalation.

Diagnostic and research activities involving clinical material, infectious cultures, and infected animals or arthropods should be performed under BSL-3 practices, containment equipment, and facilities. Due to the high risk of aerosol infection, additional personal protective equipment, including respiratory protection, should be considered for non-immune personnel. Animal work with VEE virus, EEE virus and WEE virus should be performed under ABSL-3 conditions. HEPA filtration is required on the exhaust system of laboratory and animal facilities using VEE virus.

Special Issues

Vaccines Two strains of VEE virus (TC-83 and V3526) are highly attenuated in vertebrate studies and have been either exempted (strain TC-83) or excluded (strain V3526) from select agent regulations. Because of the low level of pathogenicity, these strains may be safely handled under BSL-2 conditions without vaccination or additional personal protective equipment.

Investigational vaccine protocols have been developed to immunize at-risk laboratory or field personnel against these alphaviruses, however, the vaccines are available only on a limited basis and may be contraindicated for some personnel. Therefore, additional personal protective equipment may be warranted in lieu of vaccination. For personnel who have no neutralizing antibody titer (either by previous vaccination or natural infection), additional respiratory protection is recommended for all procedures.

Select Agent VEE virus and EEE virus are select agents requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS.

Rift Valley Fever Virus (RVFV)

RVFV was first isolated in Kenya in 1936 and subsequently shown to be endemically present in almost all areas of sub-Saharan Africa.²⁸ In periods of heavy rainfall, large epizootics occur involving primarily sheep, cattle, and human disease, although many other species are infected. The primordial vertebrate reservoir is unknown, but the introduction of large herds of highly susceptible domestic breeds in the last few decades has provided a substrate for massive virus amplification. The virus has been introduced into Egypt, Saudi Arabia, and Yemen and caused epizootics and epidemics in those countries. The largest of these was in 1977 to 1979 in Egypt with many thousands of human cases and 610 reported deaths.²⁹

Most human infections are symptomatic and the most common syndrome consists of fever, myalgia, malaise, anorexia, and other non-specific symptoms. Recovery within one to two weeks is usual but hemorrhagic fever, encephalitis, or retinitis also occurs. Hemorrhagic fever develops as the primary illness proceeds and is characterized by disseminated intravascular coagulation and hepatitis. Perhaps 2% of cases will develop this complication and the mortality is high. Encephalitis follows an apparent recovery in <1% of cases and results in a substantial mortality and sequelae. Retinal vasculitis occurs in convalescence of a substantial but not precisely known proportion of cases. The retinal lesions are often macular and permanent, leading to substantial loss of visual acuity.

Infected sheep and cattle suffer a mortality rate of 10-35%, and spontaneous abortion occurs virtually in all pregnant females. Other animals studied have lower viremia and lesser mortality but may abort. This virus is an OIE List A disease and triggers export sanctions.

Occupational Infections

The potential for infection of humans by routes other than arthropod transmission was first recognized in veterinarians performing necropsies. Subsequently, it became apparent that contact with infected animal tissues and infectious aerosols were dangerous; many infections were documented in herders, slaughterhouse workers, and veterinarians. Most of these infections resulted from exposure to blood and other tissues including aborted fetal tissues of sick animals.

There have been 47 reported laboratory infections; before modern containment and vaccination became available virtually every laboratory that began work with the virus suffered infections suggestive of aerosol transmission.^{30,31}

Natural Modes of Infection

Field studies show RVFV to be transmitted predominantly by mosquitoes, although other arthropods may be infected and transmit. Mechanical transmission also has been documented in the laboratory. Floodwater *Aedes* species are the primary vector and transovarial transmission is an important part of the maintenance cycle.³² However, many different mosquito species are implicated in horizontal transmission in field studies, and laboratory studies have shown a large number of mosquito species worldwide to be competent vectors, including North American mosquitoes.

It is currently believed that the virus passes dry seasons in the ova of flood-water *Aedes* mosquitoes. Rain allows infectious mosquitoes to emerge and feed on vertebrates. Several mosquito species can be responsible for horizontal spread, particularly in epizootic/epidemic situations. The vertebrate amplifiers are usually sheep and cattle, with two caveats; as yet undefined native African vertebrate amplifier is thought to exist and very high viremias in humans are thought to play some role in viral amplifications.³³

Transmission of diseases occurs between infected animals but is of low efficiency and virus titers in throat swabs are low. Nosocomial infection rarely if ever occurs. There are no examples of latency with RVFV, although virus may be isolated from lymphoid organs of mice and sheep for four to six weeks post-infection.

Laboratory Safety and Containment Recommendations

Concentrations of RVFV in blood and tissues of sick animals are often very high. Placenta, amniotic fluid, and fetuses from aborted domestic animals are highly infectious. Large numbers of infectious virus also are generated in cell cultures and laboratory animals.

BSL-3 practices, containment equipment and facilities are recommended for processing human or animal material in endemic zones or in non-endemic areas in emergency circumstances. Particular care should be given to stringent aerosol containment practices, autoclaving waste, decontamination of work areas, and control of egress of material from the laboratory. Other cultures, cells, or similar biological material that could potentially harbor RVFV should not be used in a RVFV laboratory and subsequently removed.

Diagnostic or research studies outside endemic areas should be performed in a BSL-3 laboratory. Personnel also must have additional respiratory protection (such as a PAPR) or be vaccinated for RVFV. In addition, the USDA may require

full BSL-3-Ag containment for research conducted in non-endemic areas in loose-housed animals. (See Appendix D.)

Special Issues

Vaccines Two apparently effective vaccines have been developed by the Department of Defense (DoD) and have been used in volunteers, laboratory staff, and field workers under investigational protocols, but neither vaccine is available at this time.

Select Agent RVFV is a select agent requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

The live-attenuated MP-12 vaccine strain is specifically exempted from the Select Agent rules. In general, BSL-2 containment is recommended for working with this strain.

The USDA may require enhanced ABSL-3, ABSL-3, or BSL-3-Ag facilities and practices for working with RVFV in the United States. (See Appendix D.) Investigators should contact the USDA for further guidance before initiating research.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS.

Table 6. Alphabetic Listing of 597 Arboviruses and Hemorrhagic Fever Viruses*

Name	Acronym	Taxonomic Status (Family or Genus)	Recommended Biosafety Level	Basis of Rating	Antigenic Group	HEPA Filtration on Lab Exhaust
Abras	ABRV	<i>Orthobunnavirus</i>	2	A7	Patois	No
Absettarov	ABSV	<i>Flavivirus</i>	4	A4	B ^f	Yes
Abu Hammad	AHV	<i>Nairovirus</i>	2	S	Dera Ghazi Khan	No
Acado	ACDV	<i>Orbivirus</i>	2	S	Corriparta	No
Acara	ACAV	<i>Orthobunyavirus</i>	2	S	Capim	No
Adelaide River	ARV	<i>Lyssavirus</i>	2	IE	Bovine Ephemeral Fever	No
African Horse sickness	AHSV	<i>Orbivirus</i>	3 ^c	A1	African Horsesickness	Yes
African Swine Fever	ASFV	<i>Asfivirus</i>	3 ^c	IE	Asfivirus	Yes

Name	Acronym	Taxonomic Status (Family or Genus)	Recommended Biosafety Level	Basis of Rating	Antigenic Group	HEPA Filtration on Lab Exhaust
Aguacate	AGUV	<i>Phlebovirus</i>	2	S	Phlebotomus Fever	No
Aino	AINOV	<i>Orthobunyavirus</i>	2	S	Simbu	No
Akabane	AKAV	<i>Orthobunyavirus</i>	3 ^c	S	Simbu	Yes
Alenquer	ALEV	<i>Phlebovirus</i>	2	IE	Phlebotomus Fever	No
Alfuy	ALFV	<i>Flavivirus</i>	2	S	B ^f	No
Alkhumra	ALKV	<i>Flavivirus</i>	4	A4	B ^f	Yes
Allpahuayo	ALLPV	<i>Arenavirus</i>	3	IE	Tacaribe	No
Almeirim	ALMV	<i>Orbivirus</i>	2	IE	Changuinola	No
Almpiwar	ALMV	<i>Rhabdoviridae</i>	2	S		No
Altamira	ALTV	<i>Orbivirus</i>	2	IE	Changuinola	No
Amapari	AMAV	<i>Arenavirus</i>	2	A5	Tacaribe	No
Ambe	AMBEV	<i>Phlebovirus</i>	2	IE		No
Ananindeua	ANUV	<i>Orthobunyavirus</i>	2	A7	Guama	No
Andasibe	ANDV	<i>Orbivirus</i>	2	A7		No
Andes	ANDV	<i>Hantavirus</i>	3 ^a	IE	Hantaan	No
Anhanga	ANHV	<i>Phlebovirus</i>	2	S	Phlebotomus Fever	No
Anhemi	AMBV	<i>Orthobunyavirus</i>	2	S	Bunyamwera	No
Anopheles A	ANAV	<i>Orthobunyavirus</i>	2	S	Anopheles A	No
Anopheles B	ANBV	<i>Orthobunyavirus</i>	2	S	Anopheles B	No
Antequera	ANTV	<i>Bunyaviridae</i>	2	IE	Resistencia	No
Apeu	APEUV	<i>Orthobunyavirus</i>	2	S	C ^f	No
Apoi	APOIV	<i>Flavivirus</i>	2	S	B ^f	No
Araguari	ARAV	Unassigned	3	IE		No
Aransas Bay	ABV	<i>Bunyaviridae</i>	2	IE	UPOLU	No
Arbia	ARBV	<i>Phlebovirus</i>	2	IE	Phlebotomus Fever	No
Arboledas	ADSV	<i>Phlebovirus</i>	2	A7	Phlebotomus Fever	No
Aride	ARIV	Unassigned	2	S		No
Ariquemes	ARQV	<i>Phlebovirus</i>	2	A7	Phlebotomus Fever	No
Arkonam	ARKV	<i>Orbivirus</i>	2	S	Ieri	No
Armero	ARMV	<i>Phlebovirus</i>	2	A7	Phlebotomus Fever	No
Aroa	AROAV	<i>Flavivirus</i>	2	S	B ^f	No
Aruac	ARUV	<i>Rhabdoviridae</i>	2	S		No

Name	Acronym	Taxonomic Status (Family or Genus)	Recommended Biosafety Level	Basis of Rating	Antigenic Group	HEPA Filtration on Lab Exhaust
Arumateua	ARMTV	<i>Orthobunyavirus</i>	2	A7		No
Arumowot	AMTV	<i>Phlebovirus</i>	2	S	Phlebotomus Fever	No
Aura	AURAV	<i>Alphavirus</i>	2	S	A ^f	No
Avalon	AVAV	<i>Nairovirus</i>	2	S	Sakhalin	No
Babahoyo	BABV	<i>Orthobunyavirus</i>	2	A7	Patois	No
Babanki	BBKV	<i>Alphavirus</i>	2	A7	A ^f	No
Bagaza	BAGV	<i>Flavivirus</i>	2	S	B ^f	No
Bahig	BAHV	<i>Orthobunyavirus</i>	2	S	Tete	No
Bakau	BAKV	<i>Orthobunyavirus</i>	2	S	Bakau	No
Baku	BAKUV	<i>Orbivirus</i>	2	S	Kemerovo	No
Bandia	BDAV	<i>Nairovirus</i>	2	S	Qalyub	No
Bangoran	BGNV	<i>Rhabdoviridae</i>	2	S		No
Bangui	BGIV	<i>Bunyaviridae</i>	2	S		No
Banzi	BANV	<i>Flavivirus</i>	2	S	B ^f	No
Barmah Forest	BFV	<i>Alphavirus</i>	2	A7	A ^f	No
Barranqueras	BQSV	<i>Bunyaviridae</i>	2	IE	Resistencia	No
Barur	BARV	<i>Rhabdoviridae</i>	2	S	Kern Canyon	No
Batai	BATV	<i>Orthobunyavirus</i>	2	S	Bunyamwera	No
Batama	BMAV	<i>Orthobunyavirus</i>	2	A7	Tete	No
Batken	BKNV	<i>Thogotovirus</i>	2	IE		No
Bauline	BAUV	<i>Orbivirus</i>	2	S	Kemerovo	No
Bear Canyon	BRCV	<i>Arenavirus</i>	3	A7		No
Bebaru	BEBV	<i>Alphavirus</i>	2	S	A ^f	No
Belem	BLMV	<i>Bunyaviridae</i>	2	IE		No
Belmont	ELV	<i>Bunyaviridae</i>	2	S		No
Belterra	BELTV	<i>Phlebovirus</i>	2	A7	Phlebotomus Fever	No
Benevides	BENV	<i>Orthobunyavirus</i>	2	A7	Capim	No
Benfica	BENV	<i>Orthobunyavirus</i>	2	A7	Capim	No
Bermejo	BMJV	<i>Hantavirus</i>	3	IE	Hantaan	No
Berrimah	BRMV	<i>Lyssavirus</i>	2	IE	Bovine Ephemeral Fever	No
Beritoga	BERV	<i>Orthobunyavirus</i>	2	S	Guama	No
Bhanja	BHAV	<i>Bunyaviridae</i>	3	S	Bhanja	No
Bimbo	BBOV	<i>Rhabdoviridae</i>	2	IE		No

Name	Acronym	Taxonomic Status (Family or Genus)	Recommended Biosafety Level	Basis of Rating	Antigenic Group	HEPA Filtration on Lab Exhaust
Bimitti	BIMV	<i>Orthobunyavirus</i>	2	S	Guama	No
Birao	BIRV	<i>Orthobunyavirus</i>	2	S	Bunyamwera	No
Bluetongue (exotic serotypes)	BTV	<i>Orbivirus</i>	3 ^c	S	Bluetongue	No
Bluetongue (non-exotic)	BTV	<i>Orbivirus</i>	2 ^c	S	Bluetongue	No
Bobaya	BOBV	<i>Bunyaviridae</i>	2	IE		No
Bobia	BIAV	<i>Orthobunyavirus</i>	2	IE	Olifantsylei	No
Boraceia	BORV	<i>Orthobunyavirus</i>	2	S	Anopheles B	No
Botambi	BOTV	<i>Orthobunyavirus</i>	2	S	Olifantsylei	No
Boteke	BTKV	<i>Vesiculovirus</i>	2	S	Vesicular Stomatitis	No
Bouboui	BOUV	<i>Flavivirus</i>	2	S	B ^f	No
Bovine Ephemeral Fever	BEFV	<i>Lyssavirus</i>	3 ^c	A1	Bovine Ephemeral Fever	No
Bozo	BOZOV	<i>Orthobunyavirus</i>	2	A7	Bunyamwera	No
Breu Branco	BRBV	<i>Orbivirus</i>	2	A7		No
Buenaventura	BUEV	<i>Phlebovirus</i>	2	IE	Phlebotomus Fever	No
Bujaru	BUJV	<i>Phlebovirus</i>	2	S	Phlebotomus Fever	No
Bunyamwera	BUNV	<i>Orthobunyavirus</i>	2	S	Bunyamwera	No
Bunyip Creek	BCV	<i>Orbivirus</i>	2	S	Palyam	No
Burg El Arab	BEAV	<i>Rhabdoviridae</i>	2	S	Matariva	No
Bushbush	BSBV	<i>Orthobunyavirus</i>	2	S	Capim	No
Bussuquara	BSQV	<i>Flavivirus</i>	2	S	B ^f	No
Buttonwillow	BUTV	<i>Orthobunyavirus</i>	2	S	Simbu	No
Bwamba	BWAV	<i>Orthobunyavirus</i>	2	S	Bwamba	No
Cabassou	CABV	<i>Alphavirus</i>	3	IE	A ^f	Yes
Cacao	CACV	<i>Phlebovirus</i>	2	S	Phlebotomus Fever	No
Cache Valley	CVV	<i>Orthobunyavirus</i>	2	S	Bunyamwera	No
Cacipacore	CPCV	<i>Flavivirus</i>	2	IE	B ^f	No
Caimito	CAIV	<i>Phlebovirus</i>	2	S	Phlebotomus Fever	No
Calchaqui	CQIV	<i>Vesiculovirus</i>	2	A7	Vesicular Stomatitis	No
California Encephalitis	CEV	<i>Orthobunyavirus</i>	2	S	California	No
Calovo	CVOV	<i>Orthobunyavirus</i>	2	S	Bunyamwera	No
Cananea	CNAV	<i>Orthobunyavirus</i>	2	IE	GUAMA	No

Name	Acronym	Taxonomic Status (Family or Genus)	Recommended Biosafety Level	Basis of Rating	Antigenic Group	HEPA Filtration on Lab Exhaust
Candiru	CDUV	<i>Phlebovirus</i>	2	S	Phlebotomus Fever	No
Caninde	CANV	<i>Orbivirus</i>	2	IE	Changuinola	No
Cano Delgadito	CADV	<i>Hantavirus</i>	3 ^a	IE	Hantaan	No
Cape Wrath	CWV	<i>Orbivirus</i>	2	S	Kemerovo	No
Capim	CAPV	<i>Orthobunyavirus</i>	2	S	Capim	No
Caraipe	CRPV	<i>Orthobunyavirus</i>	2	A7		No
Carajas	CRJV	<i>Vesiculovirus</i>	2	A7	Vesicular Stomatitis	No
Caraparu	CARV	<i>Orthobunyavirus</i>	2	S	C ^f	No
Carey Island	CIV	<i>Flavivirus</i>	2	S	B ^f	No
Catu	CATUV	<i>Orthobunyavirus</i>	2	S	Guama	No
Chaco	CHOV	<i>Rhabdoviridae</i>	2	S	Timbo	No
Chagres	CHGV	<i>Phlebovirus</i>	2	S	Phlebotomus Fever	No
Chandipura	CHPV	<i>Vesiculovirus</i>	2	S	Vesicular Stomatitis	No
Changuinola	CGLV	<i>Orbivirus</i>	2	S	Changuinola	No
Charleville	CHVV	<i>Lyssavirus</i>	2	S	Rab	No
Chenuda	CNUV	<i>Orbivirus</i>	2	S	Kemerovo	No
Chikungunya	CHIKV	<i>Alphavirus</i>	3	S	A ^f	Yes
Chilibre	CHIV	<i>Phlebovirus</i>	2	S	Phlebotomus Fever	No
Chim	CHIMV	<i>Bunyaviridae</i>	2	IE		No
Chobar Gorge	CGV	<i>Orbivirus</i>	2	S	Chobar Gorge	No
Clo Mor	CMV	<i>Nairovirus</i>	2	S	Sakhalin	No
Coastal Plains	CPV	<i>Lyssavirus</i>	2	IE	Tibrogargan	No
Cocal	COCV	<i>Vesiculovirus</i>	2	A3	Vesicular Stomatitis	No
Codajas	CDJV	<i>Orbivirus</i>	2	A7		No
Colorado Tick Fever	CTFV	<i>Coltivirus</i>	2	S	Colorado Tick Fever	No
Congo-Crimean Hemorrhagic Fever	CCHFV	<i>Nairovirus</i>	4	A6	CCHF	Yes
Connecticut	CNTV	<i>Rhabdoviridae</i>	2	IE	Sawgrass	No
Corfou	CFUV	<i>Phlebovirus</i>	2	A7	Phlebotomus Fever	No
Corriparta	CORV	<i>Orbivirus</i>	2	S	Corriparta	No
Cotia	CPV	<i>Poxviridae</i>	2	S		No
Cowbone Ridge	CRV	<i>Flavivirus</i>	2	S	B ^f	No

Name	Acronym	Taxonomic Status (Family or Genus)	Recommended Biosafety Level	Basis of Rating	Antigenic Group	HEPA Filtration on Lab Exhaust
Csiro Village	CVGV	<i>Orbivirus</i>	2	S	Palyam	No
Cuiaba	CUIV	<i>Rhabdoviridae</i>	2	S		No
Curionopolis	CRNPV	<i>Rhabdoviridae</i>	2	A7		No
Dabakala	DABV	<i>Orthobunyavirus</i>	2	A7	Olifantsylei	No
D'Aguilar	DAGV	<i>Orbivirus</i>	2	S	Palyam	No
Dakar Bat Virus	DBV	<i>Flavivirus</i>	2	S	B ^f	No
Deer Tick Virus	DRTV	<i>Flavivirus</i>	3	A7		No
Dengue Virus Type 1	DENV-1	<i>Flavivirus</i>	2	S	B ^f	No
Dengue Virus Type 2	DENV-2	<i>Flavivirus</i>	2	S	B ^f	No
Dengue Virus Type 3	DENV-3	<i>Flavivirus</i>	2	S	B ^f	No
Dengue Virus Type 4	DENV-4	<i>Flavivirus</i>	2	S	B ^f	No
Dera Ghazi Khan	DGKV	<i>Nairovirus</i>	2	S	Dera Ghazi Khan	No
Dobrava-Belgrade	DOBV	<i>Hantavirus</i>	3 ^a	IE		No
Dhori	DHOV	<i>Orthomyxoviridae</i>	2	S		No
Douglas	DOUV	<i>Orthobunyavirus</i>	3	IE	Simbu	No
Durania	DURV	<i>Phlebovirus</i>	2	A7	Phlebotomus Fever	No
Dugbe	DUGV	<i>Nairovirus</i>	3	S	Nairobi Sheep Disease	No
Eastern Equine Encephalitis	EEEV	<i>Alphavirus</i>	3 ^c	S	A ^f	No
Ebola (Including Reston)	EBOV	<i>Filovirus</i>	4	S	EBO	Yes
Edge Hill	EHV	<i>Flavivirus</i>	2	S	B ^f	No
Enseada	ENSV	<i>Bunyaviridae</i>	3	IE		No
Entebbe Bat	ENTV	<i>Flavivirus</i>	2	S	B ^f	No
Epizootic Hemorrhagic Disease	EHDV	<i>Orbivirus</i>	2	S	Epizootic Hemorrhagic Disease	No
Erve	ERVEV	<i>Bunyaviridae</i>	2	S	Thiafora	No
Esterio Real	ERV	<i>Orthobunyavirus</i>	2	IE	Patois	No
Eubenangee	EUBV	<i>Orbivirus</i>	2	S	Eubenangee	No
Everglades	EVEV	<i>Alphavirus</i>	3	S	A ^f	Yes
Eyach	EYAV	<i>Coltivirus</i>	2	S	Colorado Tick Fever	No
Farmington	FRMV	<i>Vesiculovirus</i>	2	A7		No
Flanders	FLAV	<i>Rhabdoviridae</i>	2	S	Hart Park	No

Name	Acronym	Taxonomic Status (Family or Genus)	Recommended Biosafety Level	Basis of Rating	Antigenic Group	HEPA Filtration on Lab Exhaust
Flexal	FLEV	<i>Arenavirus</i>	3	S	Tacaribe	No
Fomede	FV	<i>Orbivirus</i>	2	A7	Chobar Gorge	No
Forecariah	FORV	<i>Bunyaviridae</i>	2	A7	Bhanja	No
Fort Morgan	FMV	<i>Alphavirus</i>	2	S	A ^f	No
Fort Sherman	FSV	<i>Orthobunyavirus</i>	2	A7	Bunyamwera	No
Frijoles	FRIV	<i>Phlebovirus</i>	2	S	Phlebotomus Fever	No
Gabek Forest	GFV	<i>Phlebovirus</i>	2	A7	Phlebotomus Fever	No
Gadgets Gully	GGYV	<i>Flavivirus</i>	2	IE	B ^f	No
Gamboa	GAMV	<i>Orthobunyavirus</i>	2	S	Gamboa	No
Gan Gan	GGV	<i>Bunyaviridae</i>	2	A7	Mapputta	No
Garba	GARV	<i>Rhabdoviridae</i>	2	IE	Matariva	No
Garissa	GRSV	<i>Orthobunyavirus</i>	3	A7	Bunyamwera	No
Germiston	GERV	<i>Orthobunyavirus</i>	3		Bunyamwera	Yes
Getah	GETV	<i>Alphavirus</i>	2	A1	A ^f	No
Gomoka	GOMV	<i>Orbivirus</i>	2	S	Ieri	No
Gordil	GORV	<i>Phlebovirus</i>	2	IE	Phlebotomus Fever	No
Gossas	GOSV	<i>Rhabdoviridae</i>	2	S		No
Grand Arbaud	GAV	<i>Phlebovirus</i>	2	S	Uukuniemi	No
Gray Lodge	GLOV	<i>Vesiculovirus</i>	2	IE	Vesicular Stomatitis	No
Great Island	GIV	<i>Orbivirus</i>	2	S	Kemerovo	No
Guajara	GJAV	<i>Orthobunyavirus</i>	2	S	Capim	No
Guama	GMAV	<i>Orthobunyavirus</i>	2	S	Guama	No
Guanarito	GTOV	<i>Arenavirus</i>	4	A4	Tacaribe	Yes
Guaratuba	GTBV	<i>Orthobunyavirus</i>	2	A7	Guama	No
Guaroa	GROV	<i>Orthobunyavirus</i>	2	S	California	No
Gumbo Limbo	GLV	<i>Orthobunyavirus</i>	2	S	C ^f	No
Gurupi	GURV	<i>Orbivirus</i>	2	IE	Changuinola	No
Hantaan	HTNV	<i>Hantavirus</i>	3 ^a	S	Hantaan	No
Hanzalova	HANV	<i>Flavivirus</i>	4	A4	B ^f	Yes
Hart Park	HPV	<i>Rhabdoviridae</i>	2	S	Hart Park	No
Hazara	HAZV	<i>Nairovirus</i>	2	S	CHF-Congo	No
Highlands J	HJV	<i>Alphavirus</i>	2	S	A ^f	No
Huacho	HUAV	<i>Orbivirus</i>	2	S	Kemerovo	No

Name	Acronym	Taxonomic Status (Family or Genus)	Recommended Biosafety Level	Basis of Rating	Antigenic Group	HEPA Filtration on Lab Exhaust
Hughes	HUGV	<i>Nairovirus</i>	2	S	Hughes	No
Hypr	HYPRV	<i>Flavivirus</i>	4	S	B ^f	Yes
Iaco	IACOV	<i>Orthobunyavirus</i>	2	IE	Bunyamwera	No
Ibaraki	IBAV	<i>Orbivirus</i>	2	IE	Epizootic Hemorrhagic Disease	Yes
Icoaraci	ICOV	<i>Phlebovirus</i>	2	S	Phlebotomus Fever	No
Ieri	IERIV	<i>Orbivirus</i>	2	S	Ieri	No
Ife	IFEV	<i>Orbivirus b</i>	2	IE		No
Iguape	IGUV	<i>Flavivirus</i>	2	A7	B ^f	No
Ilesha	ILEV	<i>Orthobunyavirus</i>	2	S	Bunyamwera	No
Ilheus	ILHV	<i>Flavivirus</i>	2	S	B ^f	No
Ingwavuma	INGV	<i>Orthobunyavirus</i>	2	S	Simbu	No
Inhangapi	INHV	<i>Rhabdoviridae</i>	2	IE		No
Inini	INIV	<i>Orthobunyavirus</i>	2	IE	Simbu	No
Inkoo	INKV	<i>Orthobunyavirus</i>	2	S	California	No
Ippy	IPPYV	<i>Arenavirus</i>	2	S	Tacaribe	No
Iriri	IRRV	<i>Rhabdoviridae</i>	2	A7		No
Irituia	IRIV	<i>Orbivirus</i>	2	S	Changuinola	No
Isfahan	ISFV	<i>Vesiculovirus</i>	2	S	Vesicular Stomatitis	No
Israel Turkey Meningitis	ITV	<i>Flavivirus</i>	2 with 3 practices	S	B ^f	No
Issyk-Kul	ISKV	<i>Bunyaviridae</i>	3	IE		No
Itacaiunas	ITCNV	<i>Rhabdoviridae</i>	2	A7		No
Itaituba	ITAV	<i>Phlebovirus</i>	2	IE	Phlebotomus Fever	No
Itaporanga	ITPV	<i>Phlebovirus</i>	2	S	Phlebotomus Fever	No
Itaqui	ITQV	<i>Orthobunyavirus</i>	2	S	C ^f	No
Itimirim	ITIV	<i>Orthobunyavirus</i>	2	IE	Guama	No
Itupiranga	ITUV	<i>Orbivirus b</i>	2	IE		No
Ixcanal	IXCV	<i>Phlebovirus</i>	2	A7	Phlebotomus Fever	No
Jacareacanga	JACV	<i>Orbivirus</i>	2	IE	Corriparta	No
Jacunda	JCNV	<i>Phlebovirus</i>	2	A7	Phlebotomus Fever	No
Jamanxi	JAMV	<i>Orbivirus</i>	2	IE	Changuinola	No
Jamestown Canyon	JCV	<i>Orthobunyavirus</i>	2	S	California	No

Name	Acronym	Taxonomic Status (Family or Genus)	Recommended Biosafety Level	Basis of Rating	Antigenic Group	HEPA Filtration on Lab Exhaust
Japanaut	JAPV	<i>Orbivirus b</i>	2	S		No
Japanese Encephalitis	JEV	<i>Flavivirus</i>	3 ^c	S	B ^f	No
Jari	JARIV	<i>Orbivirus</i>	2	IE	Changuinola	No
Jatobal	JTBV	<i>Orthobunyavirus</i>	2	A7		No
Jerry Slough	JSV	<i>Orthobunyavirus</i>	2	S	California	No
Joa	JOAV	<i>Phlebovirus</i>	2	A7		No
Johnston Atoll	JAV	Unassigned	2	S	Quaranfil	No
Joinjakaka	JOIV	<i>Rhabdoviridae</i>	2	S		No
Juan Diaz	JDV	<i>Orthobunyavirus</i>	2	S	Capim	No
Jugra	JUGV	<i>Flavivirus</i>	2	S	B ^f	No
Junin	JUNV	<i>Arenavirus</i>	4	A6	Tacaribe	Yes
Jurona	JURV	<i>Vesiculovirus</i>	2	S	Vesicular Stomatitis	No
Juruaca	JRCV	<i>Picornavirus^b</i>	2	A7		No
Jutiapa	JUTV	<i>Flavivirus</i>	2	S	B ^f	No
Kadam	KADV	<i>Flavivirus</i>	2	S	B ^f	No
Kaeng Khoi	KKV	<i>Orthobunyavirus^b</i>	2	S		No
Kaikalur	KAIV	<i>Orthobunyavirus</i>	2	S	Simbu	No
Kairi	KRIV	<i>Orthobunyavirus</i>	2	A1	Bunyamwera	No
Kaisodi	KSOV	<i>Bunyaviridae</i>	2	S	Kaisodi	No
Kamese	KAMV	<i>Rhabdoviridae</i>	2	S	Hart Park	No
Kamiti River	KRV	<i>Flavivirus</i>	2	A7		No
Kammavanpettai	KMPV	<i>Orbivirus</i>	2	S		No
Kannamangalam	KANV	<i>Rhabdoviridae</i>	2	S		No
Kao Shuan	KSV	<i>Nairovirus</i>	2	S	Dera Ghazi Khan	No
Karimabad	KARV	<i>Phlebovirus</i>	2	S	Phlebotomus Fever	No
Karshi	KSIV	<i>Flavivirus</i>	2	S	B ^f	No
Kasba	KASV	<i>Orbivirus</i>	2	S	Palyam	No
Kedougou	KEDV	<i>Flavivirus</i>	2	A7	B ^f	No
Kemerovo	KEMV	<i>Orbivirus</i>	2	S	Kemerovo	No
Kern Canyon	KCV	<i>Rhabdoviridae</i>	2	S	Kern Canyon	No
Ketapang	KETV	<i>Orthobunyavirus</i>	2	S	Bakau	No
Keterah	KTRV	<i>Bunyaviridae</i>	2	S		No
Keuraliba	KEUV	<i>Rhabdoviridae</i>	2	S	Le Dantec	No

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Keystone	KEYV	<i>Orthobunyavirus</i>	2	S	California	No
Khabarovsk	KHAV	<i>Hantavirus</i>	3 ^a	IE	Hantaan	No
Khasan	KHAV	<i>Nairovirus</i>	2	IE	CCHF	No
Kimberley	KIMV	<i>Lyssavirus</i>	2	A7	Bovine Ephemeral Fever	No
Kindia	KINV	<i>Orbivirus</i>	2	A7	Palyam	No
Kismayo	KISV	<i>Bunyaviridae</i>	2	S	Bhanja	No
Klamath	KLAV	<i>Vesiculovirus</i>	2	S	Vesicular Stomatitis	No
Kokobera	KOKV	<i>Flavivirus</i>	2	S	B ^f	No
Kolongo	KOLV	<i>Lyssavirus</i>	2	S	Rab	No
Koongol	KOOV	<i>Orthobunyavirus</i>	2	S	Koongol	No
Kotonkan	KOTV	<i>Lyssavirus</i>	2	S	Rab	No
Koutango	KOUV	<i>Flavivirus</i>	3	S	B ^f	No
Kowanyama	KOWV	<i>Bunyaviridae</i>	2	S		No
Kumlinge	KUMV	<i>Flavivirus</i>	4	A4	B ^f	Yes
Kunjin	KUNV	<i>Flavivirus</i>	2	S	B ^f	No
Kununurra	KNAV	<i>Rhabdoviridae</i>	2	S		No
Kwatta	KWAV	<i>Vesiculovirus</i>	2	S	Vesicular Stomatitis	No
Kyasanur Forest Disease	KFDV	<i>Flavivirus</i>	4	S	B ^f	Yes
Kyzylgach	KYZV	<i>Alphavirus</i>	2	IE	A ^f	No
La Crosse	LACV	<i>Orthobunyavirus</i>	2	S	California	No
Lagos Bat	LBV	<i>Lyssavirus</i>	2	S	Rab	No
Laguna Negra	LANV	<i>Hantavirus</i>	3 ^a	IE		No
La Joya	LJV	<i>Vesiculovirus</i>	2	S	Vesicular Stomatitis	No
Lake Clarendon	LCV	<i>Orbivirus b</i>	2	IE		No
Landjia	LJAV	<i>Rhabdoviridae</i>	2	S		No
Langat	LGTV	<i>Flavivirus</i>	2	S	B ^f	No
Lanjan	LJNV	<i>Bunyaviridae</i>	2	S	Kaisodi	No
Las Maloyas	LMV	<i>Orthobunyavirus</i>	2	A7	Anopheles A	No
Lassa	LASV	<i>Arenavirus</i>	4	S	Tacaribe	Yes
Latino	LATV	<i>Arenavirus</i>	2	A5	Tacaribe	No
Lebombo	LEBV	<i>Orbivirus</i>	2	S		No
Lechiguanas	LECHV	<i>Hantavirus</i>	3 ^a	IE	Hantaan	No

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Le Dantec	LDV	<i>Rhabdoviridae</i>	2	S	Le Dantec	No
Lednice	LEDV	<i>Orthobunyavirus</i>	2	A7	Turlock	No
Lipovnik	LIPV	<i>Orbivirus</i>	2	S	Kemerovo	No
Llano Seco	LLSV	<i>Orbivirus</i>	2	IE	Umatilla	No
Lokern	LOKV	<i>Orthobunyavirus</i>	2	S	Bunyamwera	No
Lone Star	LSV	<i>Bunyaviridae</i>	2	S		No
Louping Ill	LIV	<i>Flavivirus</i>	3 ^c	S	B ^f	Yes
Lukuni	LUKV	<i>Orthobunyavirus</i>	2	S	Anopheles A	No
Macaua	MCAV	<i>Orthobunyavirus</i>	2	IE	Bunyamwera	No
Machupo	MACV	<i>Arenavirus</i>	4	S	Tacaribe	Yes
Madrid	MADV	<i>Orthobunyavirus</i>	2	S	C ^f	No
Maguari	MAGV	<i>Orthobunyavirus</i>	2	S	Bunyamwera	No
Mahogany Hammock	MHV	<i>Orthobunyavirus</i>	2	S	Guama	No
Main Drain	MDV	<i>Orthobunyavirus</i>	2	S	Bunyamwera	No
Malakal	MALV	<i>Lyssavirus</i>	2	S	Bovine Ephemeral	No
Manawa	MWAV	<i>Phlebovirus</i>	2	S	Uukumiemi	No
Manitoba	MNTBV	<i>Rhabdoviridae</i>	2	A7		No
Manzanilla	MANV	<i>Orthobunyavirus</i>	2	S	Simbu	No
Mapputta	MAPV	<i>Bunyaviridae</i>	2	S	Mapputta	No
Maporal	MPRLV	<i>Hantavirus</i>	3 ^a	IE	Hantaan	No
Maprik	MPKV	<i>Bunyaviridae</i>	2	S	Mapputta	No
Maraba	MARAV	<i>Vesiculovirus</i>	2	A7		No
Marajo	MRJV	Unassigned	2	IE		No
Marburg	MARV	<i>Filovirus</i>	4	S	Marburg	Yes
Marco	MCOV	<i>Rhabdoviridae</i>	2	S		No
Mariquita	MRQV	<i>Phlebovirus</i>	2	A7	Phlebotomus Fever	No
Marituba	MTBV	<i>Orthobunyavirus</i>	2	S	C ^f	No
Marrakai	MARV	<i>Orbivirus</i>	2	S	Palyam	No
Matariya	MTYV	<i>Rhabdoviridae</i>	2	S	Matariva	No
Matruh	MTRV	<i>Orthobunyavirus</i>	2	S	Tete	No
Matucare	MATV	<i>Orbivirus</i>	2	S		No
Mayaro	MAYV	<i>Alphavirus</i>	2	S	A ^f	No
Mboke	MBOV	<i>Orthobunyavirus</i>	2	A7	Bunyamwera	No
Meaban	MEAV	<i>Flavivirus</i>	2	IE	B ^f	No

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Melao	MELV	<i>Orthobunyavirus</i>	2	S	California	No
Mermet	MERV	<i>Orthobunyavirus</i>	2	S	Simbu	No
Middelburg	MIDV	<i>Alphavirus</i>	2	A1	A ^f	No
Minatitlan	MNTV	<i>Orthobunyavirus</i>	2	S	Minatitlan	No
Minnal	MINV	<i>Orbivirus</i>	2	S	Umatilla	No
Mirim	MIRV	<i>Orthobunyavirus</i>	2	S	Guama	No
Mitchell River	MRV	<i>Orbivirus</i>	2	S		No
Mobala	MOBV	<i>Arenavirus</i>	3	A7	Tacaribe	No
Modoc	MODV	<i>Flavivirus</i>	2	S	B ^f	No
Moju	MOJUV	<i>Orthobunyavirus</i>	2	S	Guama	No
Mojui Dos Campos	MDCV	<i>Orthobunyavirus</i>	2	IE		No
Mono Lake	MLV	<i>Orbivirus</i>	2	S	Kemerovo	No
Mont. Myotis Leukemia	MMLV	<i>Flavivirus</i>	2	S	B ^f	No
Monte Dourado	MDOV	<i>Orbivirus</i>	2	IE	Changuinola	No
Mopeia	MOPV	<i>Arenavirus</i>	3	A7		No
Moriche	MORV	<i>Orthobunyavirus</i>	2	S	Capim	No
Morro Bay	MBV	<i>Orthobunyavirus</i>	2	IE	California	No
Morumbi	MRMBV	<i>Phlebovirus</i>	2	A7	Phlebotomus Fever	No
Mosqueiro	MQOV	<i>Rhabdoviridae</i>	2	A7	Hart Park	No
Mossuril	MOSV	<i>Rhabdoviridae</i>	2	S	Hart Park	No
Mount Elgon Bat	MEBV	<i>Vesiculovirus</i>	2	S	Vesicular Stomatitis	No
M'Poko	MPOV	<i>Orthobunyavirus</i>	2	S	Turlock	No
Mucambo	MUCV	<i>Alphavirus</i>	3	S	A ^f	Yes
Mucura	MCRV	<i>Phlebovirus</i>	2	A7	Phlebotomus Fever	No
Munguba	MUNV	<i>Phlebovirus</i>	2	IE	Phlebotomus Fever	No
Murray Valley Encephalitis	MVEV	<i>Flavivirus</i>	3	S	B ^f	No
Murutucu	MURV	<i>Orthobunyavirus</i>	2	S	C ^f	No
Mykines	MYKV	<i>Orbivirus</i>	2	A7	Kemerovo	No
Nairobi Sheep Disease	NSDV	<i>Nairovirus</i>	3 ^c	A1	Nairobi Sheep Disease	No
Naranjal	NJLV	<i>Flavivirus</i>	2	IE	B ^f	No
Nariva	NARV	<i>Paramyxoviridae</i>	2	IE		No
Nasoule	NASV	<i>Lyssavirus</i>	2	A7	Rab	No

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Navarro	NAVV	<i>Rhabdoviridae</i>	2	S		No
Ndelle	NDEV	<i>Orthoreovirus</i>	2	A7	Ndelle	No
Ndumu	NDUV	<i>Alphavirus</i>	2	A1	A ^f	No
Negishi	NEGV	<i>Flavivirus</i>	3	S	B ^f	No
Nepuyo	NEPV	<i>Orthobunyavirus</i>	2	S	C ^f	No
Netivot	NETV	<i>Orbivirus</i>	2	A7		No
New Minto	NMV	<i>Rhabdoviridae</i>	2	IE	Sawgrass	No
Ngainingan	NGAV	<i>Lyssavirus</i>	2	S	Tibrogargan	No
Ngari d	NRIV	<i>Orthobunyavirus</i>	3	A7	Bunyamera	No
Ngoupe	NGOV	<i>Orbivirus</i>	2	A7	Eubenangee	No
Nique	NIQV	<i>Phlebovirus</i>	2	S	Phlebotomus Fever	No
Nkolbisson	NKOV	<i>Rhabdoviridae</i>	2	S	Kern Canyon	No
Nodamura	NOV	<i>Alphanodavirus</i>	2	IE		No
Nola	NOLAV	<i>Orthobunyavirus</i>	2	S	Bakau	No
Northway	NORV	<i>Orthobunyavirus</i>	2	IE	Bunyamwera	No
Ntaya	NTAV	<i>Flavivirus</i>	2	S	B ^f	No
Nugget	NUGV	<i>Orbivirus</i>	2	S	Kemerovo	No
Nyamanini	NYMV	Unassigned	2	S	Nyamanini	No
Nyando	NDV	<i>Orthobunyavirus</i>	2	S	Nyando	No
Oak Vale	OVV	<i>Rhabdoviridae</i>	2	A7		No
Odrenisrou	ODRV	<i>Phlebovirus</i>	2	A7	Phlebotomus Fever	No
Okhotskiy	OKHV	<i>Orbivirus</i>	2	S	Kemerovo	No
Okola	OKOV	<i>Bunyaviridae</i>	2	S	Tanga	No
Olifantsvlei	OLIV	<i>Orthobunyavirus</i>	2	S	Olifantsylei	No
Omo	OMOV	<i>Nairovirus</i>	2	A7	Qalyub	No
Omsk Hemorrhagic	OHFV	<i>Flavivirus</i>	4	S	B ^f	Yes
O'Nyong-Nyong	ONNV	<i>Alphavirus</i>	2	S	A ^f	Yes
Oran	ORANV	<i>Hantavirus</i>	3 ^a	IE	Hantaan	No
Oriboca	ORIV	<i>Orthobunyavirus</i>	2	S	C ^f	No
Oriximina	ORXV	<i>Phlebovirus</i>	2	IE	Phlebotomus Fever	No
Oropouche	OROV	<i>Orthobunyavirus</i>	3	S	Simbu	Yes
Orungo	ORUV	<i>Orbivirus</i>	2	S	Orungo	No
Ossa	OSSAV	<i>Orthobunyavirus</i>	2	S	C ^f	No

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Ouango	OUAV	<i>Rhabdoviridae</i>	2	IE		No
Oubangui	OUBV	<i>Poxviridae</i>	2	IE		No
Oubi	OUBIV	<i>Orthobunyavirus</i>	2	A7	Olifantsylei	No
Ourem	OURV	<i>Orbivirus</i>	2	IE	Changuinola	No
Pacora	PCAV	<i>Bunyaviridae</i>	2	S		No
Pacui	PACV	<i>Phlebovirus</i>	2	S	Phlebotomus Fever	No
Pahayokee	PAHV	<i>Orthobunyavirus</i>	2	S	Patois	No
Palma	PMAV	<i>Bunyaviridae</i>	2	IE	Bhanja	No
Palestina	PLSV	<i>Orthobunyavirus</i>	2	IE	Minatitlan	No
Palyam	PALV	<i>Orbivirus</i>	2	S	Palyam	No
Para	PARAV	<i>Orthobunyavirus</i>	2	IE	Simbu	No
Paramushir	PMRV	<i>Nairovirus</i>	2	IE	Sakhalin	No
Parana	PARV	<i>Arenavirus</i>	2	A5	Tacaribe	No
Paroo River	PRV	<i>Orbivirus</i>	2	IE		No
Pata	PATAV	<i>Orbivirus</i>	2	S		No
Pathum Thani	PTHV	<i>Nairovirus</i>	2	S	Dera Ghazi Khan	No
Patois	PATV	<i>Orthobunyavirus</i>	2	S	Patois	No
Peaton	PEAV	<i>Orthobunyavirus</i>	2	A1	Simbu	No
Pergamino	PRGV	<i>Hantavirus</i>	3 ^a	IE		No
Perinet	PERV	<i>Vesiculovirus</i>	2	A7	Vesicular Stomatitis	No
Petevo	PETV	<i>Orbivirus</i>	2	A7	Palyam	No
Phnom-Penh Bat	PPBV	<i>Flavivirus</i>	2	S	Bf	No
Pichinde	PICV	<i>Arenavirus</i>	2	A5	Tacaribe	No
Picola	PIAV	<i>Orbivirus</i>	2	IE	Wongorr	No
Piritai	PIRV	<i>Arenavirus</i>	3	IE		No
Piry	PIRYV	<i>Vesiculovirus</i>	3	S	Vesicular Stomatitis	No
Pixuna	PIXV	<i>Alphavirus</i>	2	S	A'	No
Playas	PLAV	<i>Orthobunyavirus</i>	2	IE	Bunyamwera	No
Pongola	PGAV	<i>Orthobunyavirus</i>	2	S	Bwamba	No
Ponteves	PTVV	<i>Phlebovirus</i>	2	A7	Uukuniemi	No
Potosi	POTV	<i>Orthobunyavirus</i>	2	IE	Bunyamwera	No
Powassan	POWV	<i>Flavivirus</i>	3	S	B'	No
Precarious Point	PPV	<i>Phlebovirus</i>	2	A7	Uukuniemi	No

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Pretoria	PREV	<i>Nairovirus</i>	2	S	Dera Ghazi Khan	No
Prospect Hill	PHV	<i>Hantavirus</i>	2	A8	Hantaan	No
Puchong	PUCV	<i>Lyssavirus</i>	2	S	Bovine Ephemeral fever	No
Pueblo Viejo	PVV	<i>Orthobunyavirus</i>	2	IE	Gamboia	No
Punta Salinas	PSV	<i>Nairovirus</i>	2	S	Hughes	No
Punta Toro	PTV	<i>Phlebovirus</i>	2	S	Phlebotomus Fever	No
Purus	PURV	<i>Orbivirus</i>	2	IE	Changuinola	No
Puumala	PUUV	<i>Hantavirus</i>	3 ^a	IE	Hantaan	No
Qalyub	QYBV	<i>Nairovirus</i>	2	S	Qalyub	No
Quaranfil	QRFV	Unassigned	2	S	Quaranfil	No
Radi	RADIV	<i>Vesiculovirus</i>	2	A7	Vesicular Stomatitis	No
Razdan	RAZV	<i>Bunyaviridae</i>	2	IE		No
Resistencia	RTAV	<i>Bunyaviridae</i>	2	IE	Resistencia	No
Restan	RESV	<i>Orthobunyavirus</i>	2	S	C ^f	No
Rhode Island	RHIV	<i>Rhabdoviridae</i>	2	A7		No
Rift Valley Fever	RVFV	<i>Phlebovirus</i>	3 ^c	S	Phlebotomus Fever	Yes
Rio Bravo	RBV	<i>Flavivirus</i>	2	S	B ^f	No
Rio Grande	RGV	<i>Phlebovirus</i>	2	S	Phlebotomus Fever	No
Rio Preto	RIOPV	Unassigned	2	IE		No
Rochambeau	RBUV	<i>Lyssavirus</i>	2	IE	Rab	No
Rocio	ROCV	<i>Flavivirus</i>	3	S	B ^f	Yes
Ross River	RRV	<i>Alphavirus</i>	2	S	A ^f	No
Royal Farm	RFV	<i>Flavivirus</i>	2	S	B ^f	No
Russian Spring-Summer Encephalitis	RSSEV	<i>Flavivirus</i>	4	S	B ^f	Yes
Saaremaa	SAAV	<i>Hantavirus</i>	3 ^a	IE	Hantaan	No
Sabia	SABV	<i>Arenavirus</i>	4	A4		Yes
Sabo	SABOV	<i>Orthobunyavirus</i>	2	S	Simbu	No
Saboya	SABV	<i>Flavivirus</i>	2	S	B ^f	No
Sagiyama	SAGV	<i>Alphavirus</i>	2	A1	A ^f	No
Saint-Floris	SAFV	<i>Phlebovirus</i>	2	S	Phlebotomus Fever	No
Sakhalin	SAKV	<i>Nairovirus</i>	2	S	Sakhalin	No

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Salanga	SGAV	<i>Poxviridae</i>	2	IE	SGA	No
Salehabad	SALV	<i>Phlebovirus</i>	2	S	Phlebotomus Fever	No
Salmon River	SAVV	<i>Coltivirus</i>	2	IE	Colorado Tick Fever	No
Sal Vieja	SVV	<i>Flavivirus</i>	2	A7	B ^f	No
San Angelo	SAV	<i>Orthobunyavirus</i>	2	S	California	No
Sandfly Fever, Naples	SFNV	<i>Phlebovirus</i>	2	S	Phlebotomus Fever	No
Sandfly Fever, Sicilian	SFSV	<i>Phlebovirus</i>	2	S	Phlebotomus Fever	No
Sandjimba	SJAV	<i>Lyssavirus</i>	2	S	Rab	No
Sango	SANV	<i>Orthobunyavirus</i>	2	S	Simbu	No
San Juan	SJV	<i>Orthobunyavirus</i>	2	IE	Gamboia	No
San Perlita	SPV	<i>Flavivirus</i>	2	A7	B ^f	No
Santarem	STMV	<i>Bunyaviridae</i>	2	IE		No
Santa Rosa	SARV	<i>Orthobunyavirus</i>	2	IE	Bunyamwera	No
Saraca	SRAV	<i>Orbivirus</i>	2	IE	Changuinola	No
Sathuperi	SATV	<i>Orthobunyavirus</i>	2	S	Simbu	No
Saumarez Reef	SREV	<i>Flavivirus</i>	2	IE	B ^f	No
Sawgrass	SAWV	<i>Rhabdoviridae</i>	2	S	Sawgrass	No
Sebokele	SEBV	Unassigned	2	S		No
Sedlec	SEDV	<i>Bunyaviridae</i>	2	A7		No
Seletar	SELV	<i>Orbivirus</i>	2	S	Kemerovo	No
Sembalam	SEMV	Unassigned	2	S		No
Semliki Forest	SFV	<i>Alphavirus</i>	3	A2	A ^f	No
Sena Madureira	SMV	<i>Rhabdoviridae</i>	2	IE	Timbo	No
Seoul	SEOV	<i>Hantavirus</i>	3 ^a	IE	Hantaan	No
Sepik	SEPV	<i>Flavivirus</i>	2	IE	B ^f	No
Serra Do Navio	SDNV	<i>Orthobunyavirus</i>	2	A7	California	No
Serra Norte	SRNV	<i>Phlebovirus</i>	2	A7		No
Shamonda	SHAV	<i>Orthobunyavirus</i>	2	S	Simbu	No
Shark River	SRV	<i>Orthobunyavirus</i>	2	S	Patois	No
Shokwe	SHOV	<i>Orthobunyavirus</i>	2	IE	Bunyamwera	No
Shuni	SHUV	<i>Orthobunyavirus</i>	2	S	Simbu	No
Silverwater	SILV	<i>Bunyaviridae</i>	2	S	Kaisodi	No
Simbu	SIMV	<i>Orthobunyavirus</i>	2	S	Simbu	No

Name	Acronym	Taxonomic Status (Family or Genus)	Recommended Biosafety Level	Basis of Rating	Antigenic Group	HEPA Filtration on Lab Exhaust
Simian Hemorrhagic Fever	SHFV	<i>Arterivirus</i>	2	A2	Simian Hemorrhagic Fever	No
Sindbis	SINV	<i>Alphavirus</i>	2	S	A ^f	No
Sin Nombre	SNV	<i>Hantavirus</i>	3 ^a	IE	Hantaan	No
Sixgun City	SCV	<i>Orbivirus</i>	2	S	Kemerovo	No
Slovakia	SLOV	Unassigned	3	IE		No
Snowshoe Hare	SSHV	<i>Orthobunyavirus</i>	2	S	California	No
Sokoluk	SOKV	<i>Flavivirus</i>	2	S	B ^f	No
Soldado	SOLV	<i>Nairovirus</i>	2	S	Hughes	No
Somone	SOMV	Unassigned	3	IE	Somone	No
Sororoca	SORV	<i>Orthobunyavirus</i>	2	S	Bunyamwera	No
Spondweni	SPOV	<i>Flavivirus</i>	2	S	B ^f	No
Sripur	SRIV	<i>Rhabdoviridae</i>	3	IE		No
St. Louis Encephalitis	SLEV	<i>Flavivirus</i>	3	S	B ^f	No
Stratford	STRV	<i>Flavivirus</i>	2	S	B ^f	No
Sunday Canyon	SCAV	<i>Bunyaviridae</i>	2	S		No
Tacaiuma	TCMV	<i>Orthobunyavirus</i>	2	S	Anopheles A	No
Tacaribe	TCRV	<i>Arenavirus</i>	2	A5	Tacaribe	No
Taggart	TAGV	<i>Nairovirus</i>	2	S	Sakhalin	No
Tahyna	TAHV	<i>Orthobunyavirus</i>	2	S	California	No
Tai	TAIV	<i>Bunyaviridae</i>	2	A7	Bunyamwera	No
Tamdy	TDYV	<i>Bunyaviridae</i>	2	IE		No
Tamiami	TAMV	<i>Arenavirus</i>	2	A5	Tacaribe	No
Tanga	TANV	<i>Bunyaviridae</i>	2	S	Tanga	No
Tanjong Rabok	TRV	<i>Orthobunyavirus</i>	2	S	Bakau	No
Tapara	TAPV	<i>Phlebovirus</i>	2	A7		No
Tataguine	TATV	<i>Bunyaviridae</i>	2	S		No
Tehran	THEV	<i>Phlebovirus</i>	2	A7	Phlebotomus Fever	No
Telok Forest	TFV	<i>Orthobunyavirus</i>	2	IE	Bakau	No
Tembe	TMEV	<i>Orbivirus b</i>	2	S		No
Tembusu	TMUV	<i>Flavivirus</i>	2	S	B ^f	No
Tensaw	TENV	<i>Orthobunyavirus</i>	2	S	Bunyamwera	No
Termeil	TERV	<i>Bunyavirus b</i>	2	IE		No
Tete	TETEV	<i>Orthobunyavirus</i>	2	S	Tete	No

Name	Acronym	Taxonomic Status (Family or Genus)	Recommended Biosafety Level	Basis of Rating	Antigenic Group	HEPA Filtration on Lab Exhaust
Thiafora	TFAV	<i>Bunyaviridae</i>	2	A7	Thiafora	No
Thimiri	THIV	<i>Orthobunyavirus</i>	2	S	Simbu	No
Thogoto	THOV	<i>Orthomyxoviridae</i>	2	S	Thogoto	No
Thottapalayam	TPMV	<i>Hantavirus</i>	2	S	Hantaan	No
Tibrogargan	TIBV	<i>Lyssavirus</i>	2	S	Tibrogargan	No
Tilligerry	TILV	<i>Orbivirus</i>	2	IE	Eubenangee	No
Timbo	TIMV	<i>Rhabdoviridae</i>	2	S	Timbo	No
Timboteua	TBTV	<i>Orthobunyavirus</i>	2	A7	Guama	No
Tinaroo	TINV	<i>Orthobunyavirus</i>	2	IE	Simbu	No
Tindholmur	TDMV	<i>Orbivirus</i>	2	A7	Kemerovo	No
Tlacotalpan	TLAV	<i>Orthobunyavirus</i>	2	IE	Bunyamwera	No
Tonate	TONV	<i>Alphavirus</i>	3	IE	A ^f	Yes
Topografov	TOPV	<i>Hantavirus</i>	3 ^a	IE	Hantaan	No
Toscana	TOSV	<i>Phlebovirus</i>	2	S	Phlebotomus Fever	No
Toure	TOUV	Unassigned	2	S		No
Tracambe	TRCV	<i>Orbivirus</i>	2	A7		No
Tribec	TRBV	<i>Orbivirus</i>	2	S	Kemerovo	No
Triniti	TNTV	<i>Togaviridae</i>	2	S		No
Trivittatus	TVTV	<i>Orthobunyavirus</i>	2	S	California	No
Trocaria	TROCV	<i>Alphavirus</i>	2	IE	A ^f	No
Trombetas	TRMV	<i>Orthobunyavirus</i>	2	A7		No
Trubanaman	TRUV	<i>Bunyaviridae</i>	2	S	Mapputta	No
Tsuruse	TSUV	<i>Orthobunyavirus</i>	2	S	Tete	No
Tucurui	TUCRV	<i>Orthobunyavirus</i>	2	A7		No
Tula	TULV	<i>Hantavirus</i>	2	A8		No
Tunis	TUNV	<i>Phlebovirus</i>	2	A7	Phlebotomus Fever	No
Turlock	TURV	<i>Orthobunyavirus</i>	2	S	Turlock	No
Turuna	TUAV	<i>Phlebovirus</i>	2	IE	Phlebotomus Fever	No
Tyuleniy	TYUV	<i>Flavivirus</i>	2	S	B ^f	No
Uganda S	UGSV	<i>Flavivirus</i>	2	S	B ^f	No
Umatilla	UMAV	<i>Orbivirus</i>	2	S	Umatilla	No
Umbre	UMBV	<i>Orthobunyavirus</i>	2	S	Turlock	No
Una	UNAV	<i>Alphavirus</i>	2	S	A ^f	No
Upolu	UPOV	<i>Bunyaviridae</i>	2	S	Upolu	No

Name	Acronym	Taxonomic Status (Family or Genus)	Recommended Biosafety Level	Basis of Rating	Antigenic Group	HEPA Filtration on Lab Exhaust
Uriurana	UURV	<i>Phlebovirus</i>	2	A7	Phlebotomus Fever	No
Urucuri	URUV	<i>Phlebovirus</i>	2	S	Phlebotomus Fever	No
Usutu	USUV	<i>Flavivirus</i>	2	S	B ^f	No
Utinga	UTIV	<i>Orthobunyavirus</i>	2	IE	Simbu	No
Uukuniemi	UUKV	<i>Phlebovirus</i>	2	S	Uukuniemi	No
Vellore	VELV	<i>Orbivirus</i>	2	S	Palyam	No
Venezuelan Equine Encephalitis	VEEV	<i>Alphavirus</i>	3 ^c	S	A ^f	Yes
Venkatapuram	VKTV	Unassigned	2	S		No
Vinces	VINV	<i>Orthobunyavirus</i>	2	A7	C ^f	No
Virgin River	VRV	<i>Orthobunyavirus</i>	2	A7	Anopheles A	No
Vesicular Stomatitis-Alagoas	VSAV	<i>Vesiculovirus</i>	2 ^c	S	Vesicular Stomatitis	No
Vesicular Stomatitis-Indiana	VSIV	<i>Vesiculovirus</i>	2 ^c	A3	Vesicular Stomatitis	No
Vesicular Stomatitis-New Jersey	VSNJV	<i>Vesiculovirus</i>	2 ^c	A3	Vesicular Stomatitis	No
Wad Medani	WMV	<i>Orbivirus</i>	2	S	Kemerovo	No
Wallal	WALV	<i>Orbivirus</i>	2	S	Wallal	No
Wanowrie	WANV	<i>Bunyaviridae</i>	2	S		No
Warrego	WARV	<i>Orbivirus</i>	2	S	Warrego	No
Wesselsbron	WESSV	<i>Flavivirus</i>	3 ^c	S	B ^f	Yes
Western Equine Encephalitis	WEEV	<i>Alphavirus</i>	3	S	A ^f	No
West Nile	WNV	<i>Flavivirus</i>	3	S	B ^f	No
Whataroa	WHAV	<i>Alphavirus</i>	2	S	A ^f	No
Whitewater Arroyo	WWAV	<i>Arenavirus</i>	3	IE	Tacaribe	No
Witwatersrand	WITV	<i>Bunyaviridae</i>	2	S		No
Wongal	WONV	<i>Orthobunyavirus</i>	2	S	Koongol	No
Wongorr	WGRV	<i>Orbivirus</i>	2	S	Wongorr	No
Wyeomyia	WYOV	<i>Orthobunyavirus</i>	2	S	Bunyamwera	No
Xiburema	XIBV	<i>Rhabdoviridae</i>	2	IE		No
Xingu	XINV	<i>Orthobunyavirus</i>	3			No
Yacaaba	YACV	<i>Bunyaviridae</i>	2	IE		No
Yaounde	YAOV	<i>Flavivirus</i>	2	A7	B ^f	No

Name	Acronym	Taxonomic Status (Family or Genus)	Recommended Biosafety Level	Basis of Rating	Antigenic Group	HEPA Filtration on Lab Exhaust
Yaquina Head	YHV	<i>Orbivirus</i>	2	S	Kemerovo	No
Yata	YATAV	<i>Rhabdoviridae</i>	2	S		No
Yellow Fever	YFV	<i>Flavivirus</i>	3	S	B ^f	Yes
Yogue	YOGV	<i>Bunyaviridae</i>	2	S	Yogue	No
Yoka	YOKA	<i>Poxviridae</i>	2	IE		No
Yug Bogdanovac	YBV	<i>Vesiculovirus</i>	2	IE	Vesicular Stomatitis	No
Zaliv Terpeniya	ZTV	<i>Phlebovirus</i>	2	S	Uukuniemi	No
Zegla	ZEGV	<i>Orthobunyavirus</i>	2	S	Patois	No
Zika	ZIKV	<i>Flavivirus</i>	2	S	B ^f	No
Zirqa	ZIRV	<i>Nairovirus</i>	2	S	Hughes	No

* Federal regulations, import/export requirements, and taxonomic status are subject to changes. Check with the appropriate federal agency to confirm regulations.

^a Containment requirements will vary based on virus concentration, animal species, or virus type. See the Hantavirus agent summary statement in the viral agent chapter.

^b Tentative placement in the genus.

^c These organisms are considered pathogens of significant agricultural importance by the USDA (see Appendix D) and may require additional containment (up to and including BSL-3-Ag containment). Not all strains of each organism are necessarily of concern to the USDA. Contact USDA for more information regarding exact containment/permit requirements before initiating work.

^d Alternate name for Ganjam virus.

^e Garissa virus is considered an isolate of this virus, so same containment requirements apply.

^f Antigenic groups designated A, B, and C refer to the original comprehensive and unifying serogroups established by Casals, Brown, and Whitman based on cross-reactivity among known arboviruses (2,21). Group A viruses are members of the genus *Alphavirus*, group B belong to the family *Flaviviridae*, and Group C viruses are members of the family *Bunyaviridae*.

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Section VIII-G: Toxin Agents

Botulinum Neurotoxin

Seven immunologically distinct serotypes of Botulinum neurotoxin (BoNT) have been isolated (A, B, C1, D, E, F and G). Each BoNT holotoxin is a disulfide-bonded heterodimer composed of a zinc metallo-protease “light chain” (approximately 50 kD) and a receptor binding “heavy chain” (approximately 100 kD). The heavy chain enhances cell binding and translocation of the catalytic light chain across the vesicular membrane.¹ There are also a number of important accessory proteins that can stabilize the natural toxin complex in biological systems or in buffer.

Four of the serotypes (A, B, E and, less commonly, F) are responsible for most human poisoning through contaminated food, wound infection, or infant botulism, whereas livestock may be at greater risk for poisoning with serotypes B, C1 and D.^{2,3} It is important to recognize, however, that all BoNT serotypes are highly toxic and lethal by injection or aerosol delivery. BoNT is one of the most toxic proteins known; absorption of less than one microgram (μg) of BoNT can cause severe incapacitation or death, depending upon the serotype and the route of exposure.

Diagnosis of Laboratory Exposures

Botulism is primarily clinically diagnosed through physician observations of signs and symptoms that are similar for all serotypes and all routes of intoxication.⁴ There typically is a latency of several hours to days, depending upon the amount of toxin absorbed, before the signs and symptoms of BoNT poisoning occur. The first symptoms of exposure generally include blurred vision, dry mouth and difficulty swallowing and speaking. This is followed by a descending, symmetrical flaccid paralysis, which can progress to generalized muscle weakness and respiratory failure. Sophisticated tests such as nerve conduction studies and single-fiber electromyography can support the diagnosis and distinguish it from similar neuromuscular conditions. Routine laboratory tests are of limited value because of the low levels of BoNT required to intoxicate, as well as the delay in onset of symptoms.

Laboratory Safety and Containment Recommendations

Solutions of sodium hypochlorite (0.1%) or sodium hydroxide (0.1N) readily inactivate the toxin and are recommended for decontamination of work surfaces and for spills. Additional considerations for the safe use and inactivation of toxins of biological origin are found in Appendix I. Because neurotoxin producing Clostridia species requires an anaerobic environment for growth and it is essentially not transmissible among individuals, exposure to pre-formed BoNT is the primary concern for laboratory workers. Two of the most significant hazards in working with BoNT or growing neurotoxin producing Clostridia species cultures are unintentional aerosol generation, especially during centrifugation, and accidental needle-stick. Although BoNT does not penetrate intact skin,

proteins can be absorbed through broken or lacerated skin and, therefore, BoNT samples or contaminated material should be handled with gloves.

Workers in diagnostic laboratories should be aware that neurotoxin producing Clostridia species or its spores can be stable for weeks or longer in a variety of food products, clinical samples (e.g., serum, feces) and environmental samples (e.g., soil). Stability of the toxin itself will depend upon the sterility, temperature, pH and ionic strength of the sample matrix, but useful comparative data are available from the food industry. BoNT retains its activity for long periods (at least 6-12 months) in a variety of frozen foods, especially under acidic conditions (pH 4.5-5.0) and/or high ionic strength, but the toxin is readily inactivated by heating.⁵

A documented incident of laboratory intoxication with BoNT occurred in workers who were performing necropsies on animals that had been exposed 24 h earlier to aerosolized BoNT serotype A; the laboratory workers presumably inhaled aerosols generated from the animal fur. The intoxications were relatively mild, and all affected individuals recovered after a week of hospitalization.⁶ Despite the low incidence of laboratory-associated botulism, the remarkable toxicity of BoNT necessitates that laboratory workers exercise caution during all experimental procedures.

BSL-2 practices, containment equipment, and facilities are recommended for routine dilutions, titrations or diagnostic studies with materials known to contain or have the potential to contain BoNT. Additional primary containment and personnel precautions, such as those recommended for BSL-3, should be implemented for activities with a high potential for aerosol or droplet production, or for those requiring routine handling of larger quantities of toxin.

Personnel not directly involved in laboratory studies involving botulinum toxin, such as maintenance personnel, should be discouraged from entering the laboratory when BoNT is in use until after the toxin and all work surfaces have been decontaminated. Purified preparations of toxin components, e.g. isolated BoNT "light chains" or "heavy chains," should be handled as if contaminated with holotoxin unless proven otherwise by toxicity bioassays.

Special Issues

Vaccines A pentavalent (A, B, C, D and E) botulinum toxoid vaccine (PBT) is available through the CDC as an IND. Vaccination is recommended for all personnel working in direct contact with cultures of neurotoxin producing Clostridia species or stock solutions of BoNT. Due to a possible decline in the immunogenicity of available PBT stocks for some toxin serotypes, the immunization schedule for the PBT recently has been modified to require injections at 0, 2, 12, and 24 weeks, followed by a booster at 12 months and annual boosters thereafter. Since there is a possible decline in vaccine efficacy, the current vaccine contains toxoid for only 5 of the 7 toxin types, this vaccine should not be considered as the sole means of protection and should not replace other worker protection measures.

Select Agent Botulinum toxin is a select agent requiring registration with CDC and/or USDA for possession, use, storage and/or transfer if quantities are above the minimum exemption level. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Staphylococcal Enterotoxins (SE)

SE are a group of closely related extracellular protein toxins of 23 to 29 kD molecular weight that are produced by distinct gene clusters found in a wide variety of *S. aureus* strains.^{8,9} SE belong to a large family of homologous pyrogenic exotoxins from staphylococci, streptococci and mycoplasma which are capable of causing a range of illnesses in man through pathological amplification of the normal T-cell receptor response, cytokine/lymphokine release, immunosuppression and endotoxic shock.^{9,10}

SE serotype A (SEA) is a common cause of severe gastroenteritis in humans.¹¹ It has been estimated from accidental food poisoning that exposure to as little as 0.05 to 1 µg SEA by the gastric route causes incapacitating illness.¹²⁻¹⁵ Comparative human toxicity for different serotypes of SE is largely unknown, but human volunteers exposed to 20-25 µg SE serotype B (SEB) in distilled water experienced enteritis similar to that caused by SEA.¹⁶

SE are highly toxic by intravenous and inhalation routes of exposure. By inference from accidental exposure of laboratory workers and controlled experiments with NHP, it has been estimated that inhalation of less than 1 ng/kg SEB can incapacitate more than 50% of exposed humans, and that the inhalation LD₅₀ in humans may be as low as 20 ng/kg SEB.¹⁷

Exposure of mucous membranes to SE in a laboratory setting has been reported to cause incapacitating gastrointestinal symptoms, conjunctivitis and localized cutaneous swelling.¹⁸

Diagnosis of Laboratory Exposures

Diagnosis of SE intoxication is based on clinical and epidemiologic features. Gastric intoxication with SE begins rapidly after exposure (1-4 h) and is characterized by severe vomiting, sometimes accompanied by diarrhea, but without a high fever. At higher exposure levels, intoxication progresses to hypovolemia, dehydration, vasodilatation in the kidneys, and lethal shock.¹¹

While fever is uncommon after oral ingestion, inhalation of SE causes a marked fever and respiratory distress. Inhalation of SEB causes a severe, incapacitating illness of rapid onset (3-4 h) lasting 3 to 4 days characterized by high fever, headache, and a nonproductive cough; swallowing small amounts of SE during an inhalation exposure may result in gastric symptoms as well.¹⁹

Differential diagnosis of SE inhalation may be unclear initially because the symptoms are similar to those caused by several respiratory pathogens such as influenza, adenovirus, and mycoplasma. Naturally occurring pneumonias or influenza, however, would typically involve patients presenting over a more prolonged interval of time, whereas SE intoxication tends to plateau rapidly, within a few hours. Nonspecific laboratory findings of SE inhalation include a neutrophilic leukocytosis, an elevated erythrocyte sedimentation rate, and chest X-ray abnormalities consistent with pulmonary edema.¹⁹

Laboratory confirmation of intoxication includes SE detection by immunoassay of environmental and clinical samples, and gene amplification to detect staphylococcal genes in environmental samples. SE may be undetectable in the serum at the time symptoms occur; nevertheless, a serum specimen should be drawn as early as possible after exposure. Data from animal studies suggest the presence of SE in the serum or urine is transient. Respiratory secretions and nasal swabs may demonstrate the toxin early (within 24 h of inhalation exposure). Evaluation of neutralizing antibody titers in acute and convalescent sera of exposed individuals can be undertaken, but may yield false positives resulting from pre-existing antibodies produced in response to natural SE exposure.

Laboratory Safety and Containment Recommendations

General considerations for the safe use and inactivation of toxins of biological origin are found in Appendix I. Accidental ingestion, parenteral inoculation, and droplet or aerosol exposure of mucous membranes are believed to be the primary hazards of SE for laboratory and animal-care personnel. SE are relatively stable, monomeric proteins, readily soluble in water, and resistant to proteolytic degradation and temperature fluctuations. The physical/chemical stability of SE suggests that additional care must be taken by laboratory workers to avoid exposure to residual toxin that may persist in the environment.

Active SE toxins may be present in clinical samples, lesion fluids, respiratory secretions, or tissues of exposed animals. Additional care should be taken during necropsy of exposed animals or in handling clinical stool samples because SE toxins retain toxic activity throughout the digestive tract.

Accidental laboratory exposures to SE serotype B have been reviewed.¹⁸ Documented accidents included inhalation of SE aerosols generated from pressurized equipment failure, as well as re-aerosolization of residual toxin from the fur of exposed animals. The most common cause of laboratory intoxication

with SE is expected to result from accidental self-exposure via the mucous membranes by touching contaminated hands to the face or eyes.

BSL-2 practices and containment equipment and facilities should be used when handling SE or potentially contaminated material. Because SE is highly active by the oral or ocular exposure route, the use of a laboratory coat, gloves and safety glasses is mandatory when handling toxin or toxin-contaminated solutions. Frequent and careful hand-washing and laboratory decontamination should be strictly enforced when working with SE. Depending upon a risk assessment of the laboratory operation, the use of a disposable face mask may be required to avoid accidental ingestion.

BSL-3 facilities, equipment, and practices are indicated for activities with a high potential for aerosol or droplet production and those involving the use of large quantities of SE.

Special Issues

Vaccines No approved vaccine or specific antidote is currently available for human use, but experimental, recombinant vaccines are under development.

Select Agent SE is a select agent requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Ricin Toxin

Ricin is produced in maturing seeds of the castor bean, *Ricinus communis*, which has been recognized for centuries as a highly poisonous plant for humans and livestock.²⁰ Ricin belongs to a family of ribosome inactivating proteins from plants, including abrin, modeccin, and viscumin, that share a similar overall structure and mechanism of action.²¹ The ricin holotoxin is a disulfide-bonded heterodimer composed of an A-chain (approximately 34 kD polypeptide) and a B-chain (approximately 32 kD). The A-chain is an N-glycosidase enzyme and a potent inhibitor of protein synthesis, whereas the B-chain is a relatively non-toxic lectin that facilitates toxin binding and internalization to target cells.²⁰

Ricin is much less toxic by weight than is BoNT or SE, and published case reports suggest that intramuscular or gastric ingestion of ricin is rarely fatal in adults.²² Animal studies and human poisonings suggest that the effects of ricin

depend upon the route of exposure, with inhalation and intravenous exposure being the most toxic. In laboratory mice, for example, the LD₅₀ by intravenous injection is about 5 µg/kg, whereas it is 20 mg/kg by intragastric route.^{23,24} The ricin aerosol LD₅₀ for NHP is estimated to be 10-15 µg/kg.¹⁷ The human lethal dose has not been established rigorously, but may be as low as 1-5 mg of ricin by injection or by the aerosol route (extrapolation from two species of NHP).

Diagnosis of Laboratory Exposures

The primary diagnosis is through clinical manifestations that vary greatly depending upon the route of exposure. Following inhalation exposure of NHP, there is typically a latency period of 24-72 h that may be characterized by loss of appetite and listlessness. The latency period progresses rapidly to severe pulmonary distress, depending upon the exposure level. Most of the pathology occurs in the lung and upper respiratory tract, including inflammation, bloody sputum, and pulmonary edema. Toxicity from ricin inhalation would be expected to progress despite treatment with antibiotics, as opposed to an infectious process. There would be no mediastinitis as seen with inhalation anthrax. Ricin patients would not be expected to plateau clinically as occurs after inhalation of SEB.

Gastric ingestion of ricin causes nausea, vomiting, diarrhea, abdominal cramps and dehydration. Initial symptoms may appear more rapidly following gastric ingestion (1-5 h), but generally require exposure to much higher levels of toxin compared with the inhalation route. Following intramuscular injection of ricin, symptoms may persist for days and include nausea, vomiting, anorexia, and high fever. The site of ricin injection typically shows signs of inflammation with marked swelling and induration. One case of poisoning by ricin injection resulted in fever, vomiting, irregular blood pressure, and death by vascular collapse after a period of several days; it is unclear in this case if the toxin was deposited intramuscularly or in the bloodstream.²⁵

Specific immunoassay of serum and respiratory secretions or immunohistochemical stains of tissue may be used where available to confirm a diagnosis. Ricin is an extremely immunogenic toxin, and paired acute and convalescent sera should be obtained from survivors for measurement of antibody response. Polymerase chain reaction (PCR) can detect residual castor bean DNA in most ricin preparations. Additional supportive clinical or diagnostic features, after aerosol exposure to ricin, may include the following: bilateral infiltrates on chest radiographs, arterial hypoxemia, neutrophilic leukocytosis, and a bronchial aspirate rich in protein.²⁴

Laboratory Safety and Containment Recommendations

General considerations for the safe use and inactivation of toxins of biological origin are found in Appendix I. Precautions should be extended to handling potentially contaminated clinical, diagnostic and post-mortem samples because

ricin may retain toxicity in the lesion fluids, respiratory secretions, or unfixed tissues of exposed animals.

When the ricin A-chain is separated from the B-chain and administered parenterally to animals, its toxicity is diminished by >1,000-fold compared with ricin holotoxin.²⁶ However, purified preparations of natural ricin A-chain or B-chain, as well as crude extracts from castor beans, should be handled as if contaminated by ricin until proven otherwise by bioassay.

BSL-2 practices, containment equipment and facilities are recommended, especially a laboratory coat, gloves, and respiratory protection, when handling ricin toxin or potentially contaminated materials.

Ricin is a relatively non-specific cytotoxin and irritant that should be handled in the laboratory as a non-volatile toxic chemical. A BSC (Class II, Type B1 or B2) or a chemical fume hood equipped with an exhaust HEPA filter and charcoal filter are indicated for activities with a high potential for aerosol, such as powder samples, and the use of large quantities of toxin. Laboratory coat, gloves, and full-face respirator should be worn if there is a potential for creating a toxin aerosol.

Special Issues

Vaccines No approved vaccine or specific antidote is currently available for human use, but experimental, recombinant vaccines are under development.

Select Agent Ricin toxin is a select agent requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Selected Low Molecular Weight (LMW) Toxins

LMW toxins comprise a structurally and functionally diverse class of natural poisons, ranging in size from several hundred to a few thousand daltons, that includes complex organic structures, as well as disulfide cross-linked and cyclic polypeptides. Tremendous structural diversity may occur within a particular type of LMW toxin, often resulting in incomplete toxicological or pharmacological characterization of minor isoforms. Grouping LMW toxins together has primarily been a means of distinguishing them from protein toxins with respect to key biophysical characteristics. Compared with proteins, the LMW toxins are of smaller size, which alters their filtration and biodistribution properties, are

generally more stable and persistent in the environment, and may exhibit poor water-solubility necessitating the use of organic solvent; these characteristics pose special challenges for safe handling, containment, and decontamination of LMW toxins within the laboratory.

The set of LMW toxins selected for discussion herein are employed routinely as laboratory reagents, and/or have been designated as potential public health threats by the CDC, including: T-2 mycotoxin produced by *Fusarium* fungi;^{27,28} saxitoxin and related paralytic shellfish poisons produced by dinoflagellates of the *Gonyaulax* family;²⁹ tetrodotoxin from a number of marine animals,³⁰ brevetoxin from the dinoflagellate *Ptychodiscus brevis*;³¹ palytoxin from marine coelenterates belonging to the genus *Palythoa*,³² polypeptide conotoxins α -GI (includes GIA) and α -MI from the *Conus* genus of gastropod mollusks;³³ and the monocyclic polypeptide, microcystin-LR from freshwater cyanobacteria *Microcystis aeruginosa*.³⁴

Trichothecene mycotoxins comprise a broad class of structurally complex, non-volatile sesquiterpene compounds that are potent inhibitors of protein synthesis.^{27,28} Mycotoxin exposure occurs by consumption of moldy grains, and at least one of these toxins, designated "T-2," has been implicated as a potential biological warfare agent.²⁷ T-2 is a lipid-soluble molecule that can be absorbed into the body rapidly through exposed mucosal surfaces.³⁵ Toxic effects are most pronounced in metabolically active target organs and include emesis, diarrhea, weight loss, nervous disorder, cardiovascular alterations, immunodepression, hemostatic derangement, bone marrow damage, skin toxicity, decreased reproductive capacity, and death.²⁷ The LD₅₀ for T-2 in laboratory animals ranges from 0.2 to 10 mg/kg, depending on the route of exposure, with aerosol toxicity estimated to be 20 to 50 times greater than parenteral exposure.^{17,27} Of special note, T-2 is a potent vesicant capable of directly damaging skin or corneas. Skin lesions, including frank blisters, have been observed in animals with local, topical application of 50 to 100 ng of toxin.^{27,35}

Saxitoxin and tetrodotoxin are paralytic marine toxins that interfere with normal function of the sodium channel in excitable cells of heart, muscle and neuronal tissue.³⁶ Animals exposed to 1-10 μ g/kg toxin by parenteral routes typically develop a rapid onset of excitability, muscle spasm, and respiratory distress; death may occur within 10-15 minutes from respiratory paralysis.^{29,37} Humans ingesting seafood contaminated with saxitoxin or tetrodotoxin show similar signs of toxicity, typically preceded by paresthesias of the lips, face and extremities.^{36,38}

Brevetoxins are cyclic-polyether, paralytic shellfish neurotoxins produced by marine dinoflagellates that accumulate in filter-feeding mollusks and may cause human intoxication from ingestion of contaminated seafood, or by irritation from sea spray containing the toxin.³⁶ The toxin depolarizes and opens voltage-gated sodium ion channels, effectively making the sodium channel of affected nerve or muscle cells hyper-excitable. Symptoms of human ingestion are expected to

include paresthesias of the face, throat and fingers or toes, followed by dizziness, chills, muscle pains, nausea, gastroenteritis, and reduced heart rate. Brevetoxin has a parenteral LD₅₀ of 200 µg/kg in mice and guinea pigs.³¹ Guinea pigs exposed to a slow infusion of brevetoxin develop fatal respiratory failure within 30 minutes of exposure to 20 µg/kg toxin.³⁷

Palytoxin is a structurally complex, articulated fatty acid associated with soft coral *Palythoa vestitus* that is capable of binding and converting the essential cellular Na⁺/K⁺ pump into a non-selective cation channel.^{32,39} Palytoxin is among the most potent coronary vasoconstrictors known, killing animals within minutes by cutting off oxygen to the myocardium.⁴⁰ The LD₅₀ for intravenous administration ranges from 0.025 to 0.45 µg/kg in different species of laboratory animals.⁴⁰ Palytoxin is lethal by several parenteral routes, but is about 200-fold less toxic if administered to the alimentary tract (oral or rectal) compared with intravenous administration.⁴⁰ Palytoxin disrupts normal corneal function and causes irreversible blindness at topically applied levels of approximately 400 ng/kg, despite extensive rinsing after ocular instillation.⁴⁰

Conotoxins are polypeptides, typically 10-30 amino acids long and stabilized by distinct patterns of disulfide bonds, that have been isolated from the toxic venom of marine snails and shown to be neurologically active or toxic in mammals.³³ Of the estimated >105 different polypeptides (conopeptides) present in venom of over 500 known species of *Conus*, only a few have been rigorously tested for animal toxicity. Of the isolated conotoxin subtypes that have been analyzed, at least two post-synaptic paralytic toxins, designated α-GI (includes GIA) and α-MI, have been reported to be toxic in laboratory mice with LD₅₀ values in the range of 10-100 µg/kg depending upon the species and route of exposure.

Workers should be aware, however, that human toxicity of whole or partially fractionated *Conus* venom, as well as synthetic combinations of isolated conotoxins, may exceed that of individual components. For example, untreated cases of human poisoning with venom of *C. geographus* result in an approximately 70% fatality rate, probably as a result of the presence of mixtures of various α- and µ-conotoxins with common or synergistic biological targets.^{33,41} The α-conotoxins act as potent nicotinic antagonists and the µ-conotoxins block the sodium channel.³³ Symptoms of envenomation depend upon the *Conus* species involved, generally occur rapidly after exposure (minutes), and range from severe pain to spreading numbness.⁴² Severe intoxication results in muscle paralysis, blurred or double vision, difficulty breathing and swallowing, and respiratory or cardiovascular collapse.⁴²

Microcystins (also called cyanoginosins) are monocyclic heptapeptides composed of specific combinations of L-, and D-amino acids, some with uncommon side chain structures, that are produced by various freshwater cyanobacteria.⁴³ The toxins are potent inhibitors of liver protein phosphatase type 1 and are capable of causing massive hepatic hemorrhage and death.⁴³

One of the more potent toxins in this family, microcystin-LR, has a parenteral LD₅₀ of 30 to 200 µg/kg in rodents.³⁴ Exposure to microcystin-LR causes animals to become listless and prone in the cage; death occurs in 16 to 24 h. The toxic effects of microcystin vary depending upon the route of exposure and may include hypotension and cardiogenic shock, in addition to hepatotoxicity.^{34,44}

Diagnosis of Laboratory Exposures

LMW toxins are a diverse set of molecules with a correspondingly wide range of signs and symptoms of laboratory exposure, as discussed above for each toxin. Common symptoms can be expected for LMW toxins with common mechanisms of action. For example, several paralytic marine toxins that interfere with normal sodium channel function cause rapid paresthesias of the lips, face and digits after ingestion. The rapid onset of illness or injury (minutes to hours) generally supports a diagnosis of chemical or LMW toxin exposure. Painful skin lesions may occur almost immediately after contact with T-2 mycotoxin, and ocular irritation or lesions will occur in minutes to hours after contact with T-2 or palytoxin.

Specific diagnosis of LMW toxins in the form of a rapid diagnostic test is not presently available in the field. Serum and urine should be collected for testing at specialized reference laboratories by methods including antigen detection, receptor-binding assays, or liquid chromatographic analyses of metabolites. Metabolites of several marine toxins, including saxitoxin, tetrodotoxin, and brevetoxins, are well-studied as part of routine regulation of food supplies.³⁶ Likewise, T-2 mycotoxin absorption and biodistribution has been studied, and its metabolites can be detected as late as 28 days after exposure.²⁷ Pathologic specimens include blood, urine, lung, liver, and stomach contents. Environmental and clinical samples can be tested using a gas liquid chromatography-mass spectrometry technique.

Laboratory Safety and Containment Recommendations

General considerations for the safe use and inactivation of toxins of biological origin are found in Appendix I. Ingestion, parenteral inoculation, skin and eye contamination, and droplet or aerosol exposure of mucous membranes are the primary hazards to laboratory and animal care personnel. LMW toxins also can contaminate food sources or small-volume water supplies. Additionally, the T-2 mycotoxin is a potent vesicant and requires additional safety precautions to prevent contact with exposed skin or eyes. Palytoxin also is highly toxic by the ocular route of exposure.

In addition to their high toxicity, the physical/chemical stability of the LMW toxins contribute to the risks involved in handling them in the laboratory environment. Unlike many protein toxins, the LMW toxins can contaminate surfaces as a stable, dry film that may pose an essentially indefinite contact

threat to laboratory workers. Special emphasis, therefore, must be placed upon proper decontamination of work surfaces and equipment.⁴⁵

When handling LMW toxins or potentially contaminated material, BSL-2 practices, containment, equipment and facilities are recommended, especially the wearing of a laboratory coat, safety glasses and disposable gloves; the gloves must be impervious to organic solvents or other diluents employed with the toxin.

A BSC (Class II, Type B1 or B2) or a chemical fume hood equipped with exhaust HEPA filters and a charcoal filter are indicated for activities with a high potential for aerosol, such as powder samples, and the use of large quantities of toxin. Laboratory coat and gloves should be worn if potential skin contact exists. The use of respiratory protection should be considered if potential aerosolization of toxin exists.

For LMW toxins that are not easily decontaminated with bleach solutions, it is recommended to use pre-positioned, disposable liners for laboratory bench surfaces to facilitate clean up and decontamination.

Special Issues

Vaccines No approved vaccines are currently available for human use. Experimental therapeutics for LMW toxins have been reviewed.⁴⁶

Select Agent Some LMW toxins are a select agent requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

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Section VIII-H: Prion Diseases

Transmissible spongiform encephalopathies (TSE) or prion diseases are neurodegenerative diseases which affect humans and a variety of domestic and wild animal species (Tables 7 and 8).^{1,2} A central biochemical feature of prion diseases is the conversion of normal prion protein (PrP) to an abnormal, misfolded, pathogenic isoform designated PrP^{Sc} (named for “scrapie,” the prototypic prion disease). The infectious agents that transmit prion diseases are resistant to inactivation by heat and chemicals and thus require special biosafety precautions. Prion diseases are transmissible by inoculation or ingestion of infected tissues or homogenates, and infectivity is present at high levels in brain or other central nervous system tissues, and at slightly lower levels in lymphoid tissues including spleen, lymph nodes, gut, bone marrow, and blood. Although the biochemical nature of the infectious TSE agent, or prion, is not yet proven, the infectivity is strongly associated with the presence of PrP^{Sc}, suggesting that this material may be a major component of the infectious agent.

A chromosomal gene encodes PrP^C (the cellular isoform of PrP) and no PrP genes are found in purified preparations of prions. PrP^{Sc} is derived from PrP^C by a posttranslational process whereby PrP^{Sc} acquires a high *beta*-sheet content and a resistance to inactivation by normal disinfection processes. The PrP^{Sc} is less soluble in aqueous buffers and, when incubated with protease (proteinase K), the PrP^C is completely digested (sometimes indicated by the “sensitive” superscript, PrP^{sen}) while PrP^{Sc} is resistant to protease (PrP^{res}). Neither PrP-specific nucleic acids nor virus-like particles have been detected in purified, infectious preparations.

Occupational Infections

No occupational infections have been recorded from working with prions. No increased incidence of Creutzfeldt-Jakob disease (CJD) has been found amongst pathologists who encounter cases of the disease post-mortem.

Natural Modes of Infection

The recognized diseases caused by prions are listed under Table 7 (human diseases) and Table 8 (animal diseases). The only clear risk factor for disease transmission is the consumption of infected tissues such as human brain in the case of kuru, and meat including nervous tissue in the case of bovine spongiform encephalopathy and related diseases such as feline spongiform encephalopathy. It is also possible to acquire certain diseases such as familial CJD by inheritance through the germ line.

Most TSE agents, or prions, have a preference for infection of the homologous species, but cross-species infection with a reduced efficiency is also possible. After cross-species infection there is often a gradual adaptation of specificity for the new host; however, infectivity for the original host may also be propagated for several passages over a time-span of years. The process of cross-species adaptation can also vary among individuals in the same species and the rate of adaptation and the final species specificity is difficult to predict with accuracy. Such considerations help to form the basis for the biosafety classification of different prions.

Table 7. The Human Prion Diseases

Disease	Abbreviation	Mechanism of Pathogenesis
Kuru		Infection through ritualistic cannibalism
Creutzfeldt-Jakob disease	CJD	Unknown mechanism
Sporadic CJD	sCJD	Unknown mechanism; possibly somatic mutation or spontaneous conversion of PrP ^c to PrP ^{Sc}
Variant CJD	vCJD	Infection presumably from consumption of BSE-contaminated cattle products and secondary bloodborne transmission
Familial CJD	fCJD	Germline mutations in PrP gene
Latrogenic CJD	iCJD	Infection from contaminated corneal and dural grafts, pituitary hormone, or neurosurgical equipment
Gerstmann-Sträussler-Scheinker syndrome	GSS	Germline mutations in PrP gene
Fatal familial insomnia	FFI	Germline mutations in PrP gene

Table 8. The Animal Prion Diseases

Disease	Abbreviation	Natural Host	Mechanism of Pathogenesis
Scrapie		Sheep, goats, mouflon	Infection in genetically susceptible sheep
Bovine spongiform encephalopathy	BSE	Cattle	Infection with prion-contaminated feedstuffs
Chronic wasting disease	CWD	Mule, deer, white-tailed deer, Rocky Mountain elk	Unknown mechanism; possibly from direct animal contact or indirectly from contaminated feed and water sources
Exotic ungulate encephalopathy	EUE	Nyala, greater kudu and oryx	Infection with BSE-contaminated feedstuffs
Feline spongiform encephalopathy	FSE	Domestic and wild cats in captivity	Infection with BSE-contaminated feedstuffs
Transmissible mink encephalopathy	TME	Mink (farm raised)	Infection with prion-contaminated feedstuffs

Laboratory Safety and Containment Recommendations

In the laboratory setting prions from human tissue and human prions propagated in animals should be manipulated at BSL-2. BSE prions can likewise be manipulated at BSL-2. Due to the high probability that BSE prions have been transmitted to humans, certain circumstances may require the use of BSL-3 facilities and practices. All other animal prions are manipulated at BSL-2. However, when a prion from one species is inoculated into another the resultant infected animal should be treated according to the guidelines applying to the source of the inoculum. Contact APHIS National Center for Import and Export at (301) 734-5960 for specific guidance.

Although the exact mechanism of spread of scrapie among sheep and goats developing natural scrapie is unknown, there is considerable evidence that one of the primary sources is oral inoculation with placental membranes from infected ewes. There has been no evidence for transmission of scrapie to humans, even though the disease was recognized in sheep for over 200 years. The diseases TME, BSE, FSE, and EUE are all thought to occur after the consumption of prion-infected foods.^{1,2} The exact mechanism of CWD spread among mule deer, white-tailed deer and Rocky Mountain elk is unknown. There is strong evidence that CWD is laterally transmitted and environmental contamination may play an important role in local maintenance of the disease.²

In the care of patients diagnosed with human prion disease, Standard Precautions are adequate. However, the human prion diseases in this setting

are not communicable or contagious.³ There is no evidence of contact or aerosol transmission of prions from one human to another. However, they are infectious under some circumstances, such as ritualistic cannibalism in New Guinea causing kuru, the administration of prion-contaminated growth hormone causing iatrogenic CJD, and the transplantation of prion-contaminated dura mater and corneal grafts. It is highly suspected that variant CJD can also be transmitted by blood transfusion.⁴ However, there is no evidence for bloodborne transmission of non-variant forms of CJD. Familial CJD, GSS, and FFI are all dominantly inherited prion diseases; many different mutations of the PrP gene have been shown to be genetically linked to the development of inherited prion disease. Prions from many cases of inherited prion disease have been transmitted to apes, monkeys, and mice, especially those carrying human PrP transgenes.

Special Issues

Inactivation of Prions Prions are characterized by resistance to conventional inactivation procedures including irradiation, boiling, dry heat, and chemicals (formalin, betapropiolactone, alcohols). While prion infectivity in purified samples is diminished by prolonged digestion with proteases, results from boiling in sodium dodecyl sulfate and urea are variable. Likewise, denaturing organic solvents such as phenol or chaotropic reagents such as guanidine isothiocyanate have also resulted in greatly reduced but not complete inactivation. The use of conventional autoclaves as the sole treatment has not resulted in complete inactivation of prions.⁵ Formalin-fixed and paraffin-embedded tissues, especially of the brain, remain infectious. Some investigators recommend that formalin-fixed tissues from suspected cases of prion disease be immersed for 30 min in 96% formic acid or phenol before histopathologic processing (Table 9), but such treatment may severely distort the microscopic neuropathology.

The safest and most unambiguous method for ensuring that there is no risk of residual infectivity on contaminated instruments and other materials is to discard and destroy them by incineration.⁶ Current recommendations for inactivation of prions on instruments and other materials are based on the use of sodium hypochlorite, NaOH, Environ LpH and the moist heat of autoclaving with combinations of heat and chemical being most effective (Table 9).^{5,6}

Surgical Procedures Precautions for surgical procedures on patients diagnosed with prion disease are outlined in an infection control guideline for transmissible spongiform encephalopathies developed by a consultation convened by the WHO in 1999.⁶ Sterilization of reusable surgical instruments and decontamination of surfaces should be performed in accordance with recommendations described by the CDC (www.cdc.gov) and the WHO infection control guidelines.⁶ Table 9 summarizes the key recommendations for decontamination of reusable instruments and surfaces. Contaminated disposable instruments or materials should be incinerated at 1000° C or greater.⁷

Autopsies Routine autopsies and the processing of small amounts of formalin-fixed tissues containing human prions can safely be done using Standard Precautions.⁸ The absence of any known effective treatment for prion disease demands caution. The highest concentrations of prions are in the central nervous system and its coverings. Based on animal studies, it is likely that prions are also found in spleen, thymus, lymph nodes, and intestine. The main precaution to be taken by laboratorians working with prion-infected or contaminated material is to avoid accidental puncture of the skin.³ Persons handling contaminated specimens should wear cut-resistant gloves if possible. If accidental contamination of unbroken skin occurs, the area should be washed with detergent and abundant quantities of warm water (avoid scrubbing); brief exposure (1 minute to 1N NaOH or a 1:10 dilution of bleach) can be considered for maximum safety.⁶ Additional guidance related to occupational injury are provided in the WHO infection control guidelines.⁶ Unfixed samples of brain, spinal cord, and other tissues containing human prions should be processed with extreme care in a BSL-2 facility utilizing BSL-3 practices.

Bovine Spongiform Encephalopathy Although the eventual total number of variant CJD cases resulting from BSE transmission to humans is unknown, a review of the epidemiological data from the United Kingdom indicates that BSE transmission to humans is not efficient.⁹ The most prudent approach is to study BSE prions at a minimum in a BSL-2 facility utilizing BSL-3 practices. When performing necropsies on large animals where there is an opportunity that the worker may be accidentally splashed or have contact with high-risk materials (e.g., spinal column, brain) personnel should wear full body coverage personal protective equipment (e.g., gloves, rear closing gown and face shield). Disposable plasticware, which can be discarded as a dry regulated medical waste, is highly recommended. Because the paraformaldehyde vaporization procedure does not diminish prion titers, BSCs must be decontaminated with 1N NaOH and rinsed with water. HEPA filters should be bagged out and incinerated. Although there is no evidence to suggest that aerosol transmission occurs in the natural disease, it is prudent to avoid the generation of aerosols or droplets during the manipulation of tissues or fluids and during the necropsy of experimental animals. It is further strongly recommended that impervious gloves be worn for activities that provide the opportunity for skin contact with infectious tissues and fluids.

Animal carcasses and other tissue waste can be disposed by incineration with a minimum secondary temperature of 1000°C (1832°F).⁶ Pathological incinerators should maintain a primary chamber temperature in compliance with design and applicable state regulations, and employ good combustion practices. Medical waste incinerators should comply with applicable state and federal regulations.

The alkaline hydrolysis process, using a pressurized vessel that exposes the carcass or tissues to 1 N NaOH or KOH heated to 150°C, can be used as an alternative to incineration for the disposal of carcasses and tissue.^{5,10} The process has been shown to completely inactivate TSEs (301v agent used) when used for the recommended period.

Table 9. Tissue Preparation for Human CJD and Related Diseases

1.	Histology technicians wear gloves, apron, laboratory coat, and face protection.
2.	Adequate fixation of small tissue samples (e.g., biopsies) from a patient with suspected prion disease can be followed by post-fixation in 96% absolute formic acid for 30 minutes, followed by 45 hours in fresh 10% formalin.
3.	Liquid waste is collected in a 4L waste bottle initially containing 600 ml 6N NaOH.
4.	Gloves, embedding molds, and all handling materials are disposed as regulated medical waste.
5.	Tissue cassettes are processed manually to prevent contamination of tissue processors.
6.	Tissues are embedded in a disposable embedding mold. If used, forceps are decontaminated as in Table 10.
7.	In preparing sections, gloves are worn, section waste is collected and disposed in a regulated medical waste receptacle. The knife stage is wiped with 2N NaOH, and the knife used is discarded immediately in a "regulated medical waste sharps" receptacle. Slides are labeled with "CJD Precautions." The sectioned block is sealed with paraffin.
8.	Routine staining: <ol style="list-style-type: none"> slides are processed by hand; reagents are prepared in 100 ml disposable specimen cups; after placing the cover slip on, slides are decontaminated by soaking them for 1 hour in 2N NaOH; slides are labeled as "Infectious-CJD."
9.	Other suggestions: <ol style="list-style-type: none"> disposable specimen cups or slide mailers may be used for reagents; slides for immunocytochemistry may be processed in disposable Petri dishes; equipment is decontaminated as described above or disposed as regulated medical waste.

Handling and processing of tissues from patients with suspected prion disease The special characteristics of work with prions require particular attention to the facilities, equipment, policies, and procedures involved.¹⁰ The related considerations outlined in Table 9 should be incorporated into the laboratory's risk management for this work.

Table 10. Prion Inactivation Methods for Reusable Instruments and Surfaces

1. Immerse in 1 N NaOH, heat in a gravity displacement autoclave at 121°C for 30 minutes. Clean and sterilize by conventional means.

2. Immerse in 1 N NaOH or sodium hypochlorite (20,000 ppm) for 1 hours. Transfer into water and autoclave (gravity displacement) at 121°C for 1 hour. Clean and sterilize by conventional means.

3. Immerse in 1N NaOH or sodium hypochlorite (20,000) for 1 hour. Rinse instruments with water, transfer to open pan and autoclave at 121°C (gravity displacement) or 134°C (porous load) for 1 hour. Clean and sterilize by conventional means.

4. Surfaces or heat-sensitive instruments can be treated with 2N NaOH or sodium hypochlorite (20,000 ppm) for 1 hour. Ensure surfaces remain wet for entire period, then rinse well with water. Before chemical treatment, it is strongly recommended that gross contamination of surfaces be reduced because the presence of excess organic material will reduce the strength of either NaOH or sodium hypochlorite solutions.

5. Environ LpH (EPA Reg. No. 1043-118) may be used on washable, hard, non-porous surfaces (such as floors, tables, equipment, and counters), items (such as non-disposable instruments, sharps, and sharp containers), and/or laboratory waste solutions (such as formalin or other liquids). This product is currently being used under FIFRA Section 18 exemptions in a number of states. Users should consult with the state environmental protection office prior to use.

(Adapted from www.cdc.gov ^{11,12})

Working Solutions 1 N NaOH equals 40 grams of NaOH per liter of water. Solution should be prepared daily. A stock solution of 10 N NaOH can be prepared and fresh 1:10 dilutions (1 part 10 N NaOH plus 9 parts water) used daily.

20,000 ppm sodium hypochlorite equals a 2% solution. Most commercial household bleach contains 5.25% sodium hypochlorite, therefore, make a 1:2.5 dilution (1 part 5.25% bleach plus 1.5 parts water) to produce a 20,000 ppm solution. This ratio can also be stated as two parts 5.25% bleach to three parts water. Working solutions should be prepared daily.

CAUTION: Above solutions are corrosive and require suitable personal protective equipment and proper secondary containment. These strong corrosive solutions require careful disposal in accordance with local regulations.

Precautions in using NaOH or sodium hypochlorite solutions in autoclaves: NaOH spills or gas may damage the autoclave if proper containers are not used. The use of containers with a rim and lid designed for condensation to collect and drip back into the pan is recommended. Persons who use this procedure should be cautious in handling hot NaOH solution (post-autoclave) and in avoiding potential exposure to gaseous NaOH; exercise caution during all sterilization steps; and allow the autoclave, instruments, and solutions to cool down before removal. Immersion in sodium hypochlorite bleach can cause severe damage to some instruments.

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Appendix A – Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets

Section I—Introduction

This document presents information on the design, selection, function and use of Biological Safety Cabinets (BSCs), which are the primary means of containment developed for working safely with infectious microorganisms. Brief descriptions of the facility and engineering concepts for the conduct of microbiological research are also provided. BSCs are only one part of an overall biosafety program, which requires consistent use of good microbiological practices, use of primary containment equipment and proper containment facility design. Detailed descriptions of acceptable work practices, procedures and facilities, known as Biosafety Levels 1 through 4, are presented in the CDC/NIH publication *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*.¹

BSCs are designed to provide personnel, environmental and product protection when appropriate practices and procedures are followed. Three kinds of biological safety cabinets, designated as Class I, II and III, have been developed to meet varying research and clinical needs.

Most BSCs use high efficiency particulate air (HEPA) filters in the exhaust and supply systems. The exception is a Class I BSC, which does not have HEPA filtered supply air. These filters and their use in BSCs are briefly described in Section II. Section III presents a general description of the special features of BSCs that provide varying degrees of personnel, environmental, and product protection.

Laboratory hazards and risk assessment are discussed in Section IV. Section V presents work practices, procedures and practical tips to maximize information regarding the protection afforded by the most commonly used BSCs. Facility and engineering requirements needed for the operation of each type of BSC are presented in Section VI. Section VII reviews requirements for routine annual certification of cabinet operation and integrity.

These sections are not meant to be definitive or all encompassing. Rather, an overview is provided to clarify the expectations, functions and performance of these critical primary barriers. This document has been written for the biosafety officer, laboratorian, engineer or manager who desires a better understanding of each type of cabinet; factors considered for the selection of a BSC to meet specific operational needs; and the services required to maintain the operational integrity of the cabinet.

Proper maintenance of cabinets used for work at all biosafety levels cannot be over emphasized. Biosafety Officers (BSOs) should understand that an active cabinet is a primary containment device. A BSC must be routinely inspected and tested by training personnel, following strict protocols, to verify that it is working

properly. This process is referred to as certification of the cabinet and should be performed annually.

Section II—The High Efficiency Particulate Air (HEPA) Filter and the Development of Biological Containment Devices

From the earliest laboratory-acquired typhoid infections to the hazards posed by bioterrorism, antibiotic-resistant bacteria and rapidly mutating viruses, threats to worker safety have stimulated the development and refinement of workstations in which infectious microorganisms could be safely handled. The needs to work with tissue cultures, maintain sterility of cell lines, and minimize cross-contamination have contributed to concerns regarding product integrity.

The use of proper procedures and equipment (as described in BMBL)¹ cannot be overemphasized in providing primary personnel and environmental protection. For example, high-speed blenders designed to reduce aerosol generation, needle-locking syringes, micro burners and safety centrifuge cups or sealed rotors are among the engineered devices that protect laboratory workers from biological hazards. An important piece of safety equipment is the biological safety cabinet in which manipulations of infectious microorganisms are performed.

Background

Early prototype clean air cubicles were designed to protect the materials being manipulated from environmental or worker-generated contamination rather than to protect the worker from the risks associated with the manipulation of potentially hazardous materials. Filtered air was blown across the work surface directly at the worker. Therefore, these cubicles could not be used for handling infectious agents because the worker was in a contaminated air stream.

To protect the worker during manipulations of infectious agents, a small workstation was needed that could be installed in existing laboratories with minimum modification to the room. The earliest designs for primary containment devices were essentially non-ventilated “boxes” built of wood and later of stainless steel, within which simple operations such as weighing materials could be accomplished.²

Early versions of ventilated cabinets did not have adequate or controlled directional air movement. They were characterized by mass airflow into the cabinets albeit with widely varying air volumes across openings. Mass airflow into cabinet drew “contaminated” air away from the laboratory worker. This was the forerunner of the Class I BSC. However, since the air was unfiltered, the cabinet was contaminated with environmental microorganisms and other undesirable particulate matter.

Control of airborne particulate materials became possible with the development of filters, which efficiently removed microscopic contaminants from the air. The HEPA filter was developed to create dust-free work environments (e.g., “clean rooms” and “clean benches”) in the 1940s.²

HEPA filters remove the most penetrating particle size (MPPS) of 0.3 μm with an efficiency of at least 99.97%. Particles both larger and smaller than the MPPS are removed with greater efficiency. Bacteria, spores and viruses are removed from the air by these filters. HEPA filter efficiency and the mechanics of particle collection by these filters have been studied and well-documented^{3,4} therefore only a brief description is included here.

The typical HEPA filter medium is a single sheet of borosilicate fibers treated with a wet-strength water-repellant binder. The filter medium is pleated to increase the overall surface area inside the filter frames and the pleats are often divided by corrugated aluminum separators (Figure 1). The separators prevent the pleats from collapsing in the air stream and provide a path for airflow. Alternate designs providing substitutions for the aluminum separators may also be used. The filter is glued into a wood, metal or plastic frame. Careless handling of the filter (e.g., improper storage or dropping) can damage the medium at the glue joint and cause tears or shifting of the filter resulting in leaks in the medium. This is the primary reason why filter integrity must be tested when a BSC is installed initially and each time it is moved or relocated. (See Section VII.)

Various types of containment and clean air devices incorporate the use of HEPA filters in the exhaust and/or supply air system to remove airborne particulate material. Depending on the configuration of these filters and the direction of the airflow, varying degrees of personnel, environmental and product protection can be achieved.⁵ Section V describes the proper practices and procedures necessary to maximize the protection afforded by the device.

Section III—Biological Safety Cabinets

The similarities and differences in protection offered by the various classes of BSCs are reflected in Table 1. Please also refer to Table 2 and Section IV for further considerations pertinent to BSC selection and risk assessment.

The Class I BSC

The Class I BSC provides personnel and environmental protection, but no product protection. It is similar in terms of air movement to a chemical fume hood, but has a HEPA filter in the exhaust system to protect the environment (Figure 2). In the Class I BSC, unfiltered room air is drawn in through the work opening and across the work surface. Personnel protection is provided by this inward airflow as long as a minimum velocity of 75 linear feet per minute (lfm) is maintained⁶ through the front opening. Because product protection is provided by the Class II BSCs, general usage of the Class I BSC has declined. However, in many cases, Class I BSCs are used specifically to enclose equipment (e.g., centrifuges, harvesting equipment or small fermenters), or procedures with potential to generate aerosols (e.g., cage dumping, culture aeration or tissue homogenation).

The classical Class I BSC is hard-ducted (i.e., direct connection) to the building exhaust system and the building exhaust fan provides the negative pressure necessary to draw room air into the cabinet. Cabinet air is drawn through a HEPA filter as it enters the cabinet exhaust plenum. A second HEPA filter may be installed at the terminal end of the building exhaust system prior to the exhaust fan.

Some Class I BSCs are equipped with an integral exhaust fan. The cabinet exhaust fan must be interlocked with the building exhaust fan. In the event that the building exhaust fan fails, the cabinet exhaust fan must turn off so that the building exhaust ducts are not pressurized. If the ducts are pressurized and the HEPA filter has developed a leak, contaminated air could be discharged into other parts of the building or the environment. The use of two filters in the cabinet increases the static pressure on the fan.

A panel with openings to allow access for the hands and arms to the work surface can be added to the Class I cabinet. The restricted opening results in increased inward air velocity, increasing worker protection. For added safety, arm-length gloves can be attached to the panel. Makeup air is then drawn through an auxiliary air supply opening (which may contain a filter) and/or around a loose-fitting front panel.

Some Class I models used for animal cage changing are designed to allow recirculation of air into the room after HEPA filtration and may require more frequent filter replacement due to filter loading and odor from organic materials captured on the filter. This type of Class I BSC should be certified annually for sufficient airflow and filter integrity.

The Class II BSC

As biomedical researchers began to use sterile animal tissue and cell culture systems, particularly for the propagation of viruses, cabinets were needed that also provided product protection. In the early 1960s, the “laminar flow” principle evolved. Unidirectional air moving at a fixed velocity along parallel lines was demonstrated to reduce turbulence resulting in predictable particle behavior. Biocontainment technology also incorporated this laminar flow principle with the use of the HEPA filter to aid in the capture and removal of airborne contaminants from the air stream.⁷ This combination of technologies serves to help protect the laboratory worker from potentially infectious aerosols⁴ generated within the cabinet and provides necessary product protection, as well. Class II BSCs are partial barrier systems that rely on the directional movement of air to provide containment. As the air curtain is disrupted (e.g., movement of materials in and out of a cabinet, rapid or sweeping movement of the arms) the potential for contaminant release into the laboratory work environment is increased, as is the risk of product contamination.

The Class II (Types A1, A2, B1 and B2)⁸ BSCs provide personnel, environmental and product protection. Airflow is drawn into the front grille of

the cabinet, providing personnel protection. In addition, the downward flow of HEPA-filtered air provides product protection by minimizing the chance of cross-contamination across the work surface of the cabinet. Because cabinet exhaust air is passed through a certified HEPA filter, it is particulate-free (environmental protection), and may be recirculated to the laboratory (Type A1 and A2 BSCs) or discharged from the building via a canopy or “thimble” connected to the building exhaust. Exhaust air from Types B1 and B2 BSCs must be discharged directly to the outdoors via a hard connection.

HEPA filters are effective at trapping particulates and thus infectious agents but do not capture volatile chemicals or gases. Only Type A2-exhausted or Types B1 and B2 BSCs exhausting to the outside should be used when working with volatile, toxic chemicals, but amounts must be limited (Table 2).

All Class II cabinets are designed for work involving microorganisms assigned to biosafety levels 1, 2, 3 and 4.¹ Class II BSCs provide the microbe-free work environment necessary for cell culture propagation and also may be used for the formulation of nonvolatile antineoplastic or chemotherapeutic drugs.⁹ Class II BSCs may be used with organisms requiring BSL-4 containment in a BSL-4 suit laboratory by a worker wearing a positive pressure protective suit.

1. *The Class II, Type A1 BSC:* An internal fan (Figure 3) draws sufficient room air through the front grille to maintain a minimum calculated or measured average inflow velocity of at least 75 lfm at the face opening of the cabinet. The supply air flows through a HEPA filter and provides particulate-free air to the work surface. Airflow provided in this manner reduces turbulence in the work zone and minimizes the potential for cross-contamination.

The downward moving air “splits” as it approaches the work surface; the fan⁶ draws part of the air to the front grille and the remainder to the rear grille. Although there are variations among different cabinets, this split generally occurs about halfway between the front and rear grilles and two to six inches above the work surface.

The air is drawn through the front and rear grilles by a fan pushed into the space between the supply and exhaust filters. Due to the relative size of these two filters, approximately 30% of the air passes through the exhaust HEPA filter and 70% recirculates through the supply HEPA filter back into the work zone of the cabinet. Most Class II, Type A1 and A2 cabinets have dampers to modulate this division of airflow.

A Class II Type A1 BSC is not to be used for work involving volatile toxic chemicals. The buildup of chemical vapors in the cabinet (by recirculated air) and in the laboratory (from exhaust air) could create health and safety hazards (See Section IV).

It is possible to exhaust the air from a Type A1 or A2 cabinet outside of the building. However, it must be done in a manner that does not alter the balance of the cabinet exhaust system, thereby disturbing the internal cabinet airflow. The proper method of connecting a Type A1 or A2 cabinet to the building exhaust system is through use of a canopy hood,^{8,10} which provides a small opening or air gap (usually 1 inch) around the cabinet exhaust filter housing (Figure 4). The airflow of the building exhaust must be sufficient to maintain the flow of room air into the gap between the canopy unit and the filter housing. The canopy must be removable or be designed to allow for operational testing of the cabinet. (See Section VI.) Class II Type A1 or A2 cabinets should never be hard-ducted to the building exhaust system.⁸ Fluctuations in air volume and pressure that are common to all building exhaust systems sometimes make it difficult to match the airflow requirements of the cabinet.

2. *The Class II, Type B1 BSC:* Some biomedical research requires the use of small quantities of hazardous chemicals, such as organic solvents or carcinogens. Carcinogens used in cell culture or microbial systems require both biological and chemical containment.¹¹

The Class II, Type B cabinet originated with the National Cancer Institute (NCI)-designed Type 212 (later called Type B) BSC (Figure 5A), and was designed for manipulations of minute quantities of hazardous chemicals with *in vitro* biological systems. The NSF International NSF/ANSI Standard 49—2007 definition of Type B1 cabinets⁹ includes this classic NCI design Type B, and cabinets without supply HEPA filters located immediately below the work surface (Figure 5B), and/or those with exhaust/recirculation down flow splits other than exactly 70/30%.

The cabinet supply blowers draw room air (plus a portion of the cabinet's recirculated air) through the front grille and through the supply HEPA filters located immediately below the work surface. This particulate-free air flows upward through a plenum at each side of the cabinet and then downward to the work area through a backpressure plate. In some cabinets, there is an additional supply HEPA filter to remove particulates that may be generated by the blower-motor system.

Room air is drawn through the face opening of the cabinet at a minimum measured inflow velocity of 100 fpm. As with the Type A1 and A2 cabinets, there is a split in the down-flowing air stream just above the work surface. In the Type B1 cabinet, approximately 70 percent of the down flow air exits through the rear grille, passes through the exhaust HEPA filter, and is discharged from the building. The remaining 30 percent of the down flow air is drawn through the front grille. Since the air that flows to the rear grille is discharged into the exhaust system, activities that may

generate hazardous chemical vapors or particulates should be conducted toward the rear of the cabinetwork area.¹³

Type B1 cabinets must be hard-ducted, preferably to a dedicated, independent exhaust system. As indicated earlier, fans for laboratory exhaust systems should be located at the terminal end of the ductwork to avoid pressuring the exhaust ducts. A failure in the building exhaust system may not be apparent to the user, as the supply blowers in the cabinet will continue to operate. A pressure-independent monitor and alarm should be installed to provide warning and shut off the BSC supply fan, should failure in exhaust airflow occur. Since this feature is not supplied by all cabinet manufacturers, it is prudent to install a sensor such as a flow monitor and alarm in the exhaust system as necessary. To maintain critical operations, laboratories using Type B1 BSCs should connect the exhaust blower to the emergency power supply.

3. *The Class II, Type B2 BSC:* This BSC is a total-exhaust cabinet; no air is recirculated within it (Figure 6). This cabinet provides simultaneous primary biological and chemical (small quantity) containment. Consideration must be given to the chemicals used in BSCs as some chemicals can destroy the filter medium, housings and/or gaskets causing loss of containment. The supply blower draws either room or outside air in at the top of the cabinet, passes it through a HEPA filter and down into the work area of the cabinet. The building exhaust system draws air through both the rear and front grills, capturing the supply air plus the additional amount of room air needed to produce a minimum calculated or measured inflow face velocity of 100 lfm. All air entering this cabinet is exhausted, and passes through a HEPA filter (and perhaps some other air-cleaning device such as a carbon filter if required for the work being performed) prior to discharge to the outside. This cabinet exhausts as much as 1200 cubic feet per minute of conditioned room air making this cabinet expensive to operate. The higher static air pressure required to operate this cabinet also results in additional costs associated with heavier gauge ductwork and higher capacity exhaust fan. Therefore, the need for the Class II, Type B2 should be justified by the research to be conducted.

Should the building exhaust system fail, the cabinet will be pressurized, resulting in a flow of air from the work area back into the laboratory. Cabinets built since the early 1980's usually have an interlock system, installed by the manufacturer, to prevent the supply blower from operating whenever the exhaust flow is insufficient; systems can be retrofitted if necessary. Exhaust air movement should be monitored by a pressure-independent device, such as a flow monitor.

4. *The Class II, Type A2 BSC (Formerly called A/B3):* Only when this BSC (Figure 7) is ducted to the outdoors does it meet the requirements of the

former Class II Type B3.⁸ The Type A2 cabinet has a minimum calculated or measured inflow velocity of 100 lfm. All positive pressure contaminated plenums within the cabinet are surrounded by a negative air pressure plenum thus ensuring that any leakage from a contaminated plenum will be drawn into the cabinet and not released to the environment. Minute quantities of volatile toxic chemicals or radionuclides can be used in a Type A2 cabinet only if it exhausts to the outside via a properly functioning canopy connection.⁸

5. *Special Applications:* Class II BSCs can be modified to accommodate special tasks. For example, the front sash can be modified by the manufacturer to accommodate the eyepieces of a microscope. The work surface can be designed to accept a carboy, a centrifuge or other equipment that may require containment. A rigid plate with openings for the arms can be added if needed. Good cabinet design, microbiological aerosol tracer testing of the modification and appropriate certification (see Section VII) are required to ensure that the basic systems operate properly after modification. Maximum containment potential is achieved only through strict adherence to proper practices and procedures (see Section V).

The Class III BSC

The Class III BSC (Figure 8) was designed for work with highly infectious microbiological agents and for the conduct of hazardous operations and provides maximum protection for the environment and the worker. It is a gas-tight (no leak greater than 1×10^{-7} cc/sec with 1% test gas at 3 inches pressure Water Gauge¹⁴) enclosure with a non-opening view window. Access for passage of materials into the cabinet is through a dunk tank, that is accessible through the cabinet floor, or double-door pass-through box (e.g., an autoclave) that can be decontaminated between uses. Reversing that process allows materials to be removed from the Class III BSC safely. Both supply and exhaust air are HEPA filtered on a Class III cabinet. Exhaust air must pass through two HEPA filters, or a HEPA filter and an air incinerator, before discharge directly to the outdoors. Class III cabinets are not exhausted through the general laboratory exhaust system. Airflow is maintained by an exhaust system exterior to the cabinet, which keeps the cabinet under negative pressure (minimum of 0.5 inches of water gauge.)

Long, heavy-duty rubber gloves are attached in a gas-tight manner to ports in the cabinet to allow direct manipulation of the materials isolated inside. Although these gloves restrict movement, they prevent the user's direct contact with the hazardous materials. The trade-off is clearly on the side of maximizing personal safety. Depending on the design of the cabinet, the supply HEPA filter provides particulate-free, albeit somewhat turbulent, airflow within the work environment. Laminar airflow is not a characteristic of a Class III cabinet.

Several Class III BSCs can be joined together in a “line” to provide a larger work area. Such cabinet lines are custom-built; the equipment installed in the cabinet line (e.g., refrigerators, small elevators, shelves to hold small animal cage racks, microscopes, centrifuges, incubators) is generally custom-built as well.

Horizontal Laminar Flow “Clean Bench”

Horizontal laminar flow “clean benches” (Figure 9A) are not BSCs. These pieces of equipment discharge HEPA-filtered air from the back of the cabinet across the work surface and toward the user. These devices only provide product protection. They can be used for certain clean activities, such as the dust-free assembly of sterile equipment or electronic devices. Clean benches should never be used when handling cell culture materials, drug formulations, potentially infectious materials, or any other potentially hazardous materials. The worker will be exposed to the materials being manipulated on the clean bench potentially resulting in hypersensitivity, toxicity or infection depending on the materials being handled. Horizontal airflow “clean benches” must never be used as a substitute for a biological safety cabinet. Users must be aware of the differences between these two devices.

Vertical Flow “Clean Bench”

Vertical flow clean benches (Figure 9B) also are not BSCs. They may be useful, for example, in hospital pharmacies when a clean area is needed for preparation of intravenous solutions. While these units generally have a sash, the air is usually discharged into the room under the sash, resulting in the same potential problems presented by the horizontal laminar flow clean benches. These benches should never be used for the manipulation of potentially infectious or toxic materials or for preparation of antineoplastic agents.

Section IV—Other Laboratory Hazards and Risk Assessment

Primary containment is an important strategy in minimizing exposure to the many chemical, radiological and biological hazards encountered in the laboratory. An overview is provided, in Table 2, of the various classes of BSCs, the level of containment afforded by each and the appropriate risk assessment considerations. Microbiological risk assessment is addressed in depth in BMBL.¹

Working with Chemicals in BSCs

Work with infectious microorganisms often requires the use of various chemical agents, and many commonly used chemicals vaporize easily. Therefore, evaluation of the inherent hazards of the chemicals must be part of the risk assessment when selecting a BSC. Flammable chemicals should not be used in Class II, Type A1 or A2 cabinets since vapor buildup inside the cabinet presents a fire hazard. In order to determine the greatest chemical concentration, which might be entrained in the air stream following an accident or spill, it is necessary

to evaluate the quantities to be used. Mathematical models are available to assist in these determinations.¹³ For more information regarding the risks associated with exposure to chemicals, the reader should consult the Threshold Limit Values (TLVs) for various chemical substances established by the American Conference of Governmental Industrial Hygienists.¹⁵

The electrical systems of Class II BSCs are not spark-proof. Therefore, a chemical concentration approaching the lower explosive limits of the compound must be prohibited. Furthermore, since non-exhausted Class II, Type A1 and A2 cabinets return chemical vapors to the cabinetwork space and the room, they may expose the operator and other room occupants to toxic chemical vapors.

A chemical fume hood should be used for procedures using volatile chemicals instead of a BSC. Chemical fume hoods are connected to an independent exhaust system and operate with single-pass air discharged, directly or through a manifold, outside the building. They may also be used when manipulating chemical carcinogens.¹¹ When manipulating small quantities of volatile toxic chemicals required for use in microbiological studies, Class I and Class II (Type B2) BSCs, exhausted to the outdoors, can be used. The Class II, Type B1 and A2 canopy-exhausted cabinets may be used with minute or tracer quantities of nonvolatile toxic chemicals.⁸

Many liquid chemicals, including nonvolatile antineoplastic agents, chemotherapeutic drugs and low-level radionuclides, can be safely handled inside Class II, Type A cabinets.⁹ Class II BSCs should not be used for labeling of biohazardous materials with radioactive iodine. Hard-ducted, ventilated containment devices incorporating both HEPA and charcoal filters in the exhaust systems are necessary for the conduct of this type of work (Figure 10).

Many virology and cell culture laboratories use diluted preparations of chemical carcinogens^{11,16} and other toxic substances. Prior to maintenance, careful evaluation must be made of potential problems associated with decontaminating the cabinet and the exhaust system. Air treatment systems, such as a charcoal filter in a bag-in/bag-out housing,¹⁷ (Figure 13) may be required so that discharged air meets applicable emission regulations.

National Sanitation Foundation (NSF)/ANSI Standard 49—2007⁸ requires biologically-contaminated ducts and plenums of Class II, Type A2 and B cabinets be maintained under negative air pressure, or surrounded by negative pressure ducts and plenums.

Radiological Hazards in the BSC

As indicated above, volatile radionuclides such as I¹²⁵ should not be used within Class II BSCs. When using nonvolatile radionuclides inside a BSC, the same hazards exist as if working with radioactive materials on the bench top. Work that has the potential for splatter or creation of aerosols can be done within the BSC.

Radiologic monitoring must be performed. A straight, vertical (not sloping) beta shield may be used inside the BSC to provide worker protection. A sloping shield can disrupt the air curtain and increase the possibility of contaminated air being released from the cabinet. A radiation safety professional should be contacted for specific guidance.

Risk Assessment

The potential for adverse events must be evaluated to eliminate or reduce to the greatest extent possible worker exposure to infectious organisms and to prevent release to the environment. Agent summary statements detailed in BMBL¹ provide data for microorganisms known to have caused laboratory-associated infections that may be used in protocol-driven risk assessment. Through the process of risk assessment, the laboratory environment and the work to be conducted are evaluated to identify hazards and develop interventions to ameliorate risks.

A properly certified and operational BSC is an effective engineering control (see Section VI) that must be used in concert with the appropriate practices, procedures and other administrative controls to further reduce the risk of exposure to potentially infectious microorganisms. Suggested work practices and procedures for minimizing risks when working in a BSC are detailed in the next section.

Section V — BSC Use by the Investigator: Work Practices and Procedures

Preparing for Work Within a Class II BSC

Preparing a written checklist of materials necessary for a particular activity and placing necessary materials in the BSC before beginning work serves to minimize the number and extent of air curtain disruptions compromising the fragile air barrier of the cabinet. The rapid movement of a worker's arms in a sweeping motion into and out of the cabinet will disrupt the air curtain and compromise the partial containment barrier provided by the BSC. Moving arms in and out slowly, perpendicular to the face opening of the cabinet will reduce this risk. Other personnel activities in the room (e.g., rapid movements near the face of the cabinet, walking traffic, room fans, open/closing room doors) may also disrupt the cabinet air barrier.⁶

Laboratory coats should be worn buttoned over street clothing; latex, vinyl, nitrile or other suitable gloves are worn to provide hand protection. Increasing levels of PPE may be warranted as determined by an individual risk assessment. For example, a solid front, back-closing laboratory gown provides better protection of personal clothing than a traditional laboratory coat and is a recommended practice at BSL-3.

Before beginning work, the investigator should adjust the stool height so that his/her face is above the front opening. Manipulation of materials should be delayed for approximately one minute after placing the hands/arms inside the

cabinet. This allows the cabinet to stabilize, to “air sweep” the hands and arms, and to allow time for turbulence reduction. When the user’s arms rest flatly across the front grille, occluding the grille opening, room air laden with particles may flow directly into the work area, rather than being drawn down through the front grille. Raising the arms slightly will alleviate this problem. The front grille must not be blocked with toweling, research notes, discarded plastic wrappers, pipetting devices, etc. All operations should be performed on the work surface at least four inches in from the front grille. If there is a drain valve under the work surface, it should be closed prior to beginning work in the BSC.

Materials or equipment placed inside the cabinet may cause disruption of the airflow, resulting in turbulence, possible cross-contamination and/or breach of containment. Extra supplies (e.g., additional gloves, culture plates or flasks, culture media) should be stored outside the cabinet. Only the materials and equipment required for the immediate work should be placed in the BSC.

BSCs are designed for 24-hour per day operation and some investigators find that continuous operation helps to control the laboratory’s level of dust and other airborne particulates. Although energy conservation may suggest BSC operation only when needed, especially if the cabinet is not used routinely, room air balance is an overriding consideration. Air discharged through ducted BSCs must be considered in the overall air balance of the laboratory.

If the cabinet has been shut down, the blowers should be operated at least four minutes before beginning work to allow the cabinet to “purge.” This purge will remove any suspended particulates in the cabinet. The work surface, the interior walls (except the supply filter diffuser), and the interior surface of the window should be wiped with 70% ethanol (EtOH), a 1:100 dilution of household bleach (i.e., 0.05% sodium hypochlorite), or other disinfectant as determined by the investigator to meet the requirements of the particular activity. When bleach is used, a second wiping with sterile water is needed to remove the residual chlorine, which may eventually corrode stainless steel surfaces. Wiping with non-sterile water may recontaminate cabinet surfaces, a critical issue when sterility is essential (e.g., maintenance of cell cultures).

Similarly, the surfaces of all materials and containers placed into the cabinet should be wiped with 70% EtOH to reduce the introduction of contaminants to the cabinet environment. This simple step will reduce introduction of mold spores and thereby minimize contamination of cultures. Further reduction of microbial load on materials to be placed or used in BSCs may be achieved by periodic decontamination of incubators and refrigerators.

Material Placement Inside the BSC

Plastic-backed absorbent toweling can be placed on the work surface but not on the front or rear grille openings. The use of toweling facilitates routine cleanup

and reduces splatter and aerosol generation¹⁹ during an overt spill. It can be folded and placed in a biohazard bag or other appropriate receptacle when work is completed.

All materials should be placed as far back in the cabinet as practical, toward the rear edge of the work surface and away from the front grille of the cabinet (Figure 11). Similarly, aerosol-generating equipment (e.g., vortex mixers, tabletop centrifuges) should be placed toward the rear of the cabinet to take advantage of the air split described in Section III. Bulky items such as biohazard bags, discard pipette trays and vacuum collection flasks should be placed to one side of the interior of the cabinet. If placing those items in the cabinet requires opening the sash, make sure that the sash is returned to its original position before work is initiated. The correct sash position (usually 8" or 10" above the base of the opening) should be indicated on the front of the cabinet. On most BSCs, an audible alarm will sound if the sash is in the wrong position while the fan is operating.

Certain common practices interfere with the operation of the BSC. The biohazard collection bag should not be taped to the outside of the cabinet. Upright pipette collection containers should not be used in BSCs nor placed on the floor outside the cabinet. The frequent inward/outward movement needed to place objects in these containers is disruptive to the integrity of the cabinet air barrier and can compromise both personnel and product protection. Only horizontal pipette discard trays containing an appropriate chemical disinfectant should be used within the cabinet. Furthermore, potentially contaminated materials should not be brought out of the cabinet until they have been surface decontaminated. Alternatively, contaminated materials can be placed into a closable container for transfer to an incubator, autoclave or another part of the laboratory.

Operations Within a Class II BSC

Laboratory Hazards

Many procedures conducted in BSCs may create splatter or aerosols. Good microbiological techniques should always be used when working in a BSC. For example, techniques used to reduce splatter and aerosol generation will also minimize the potential for personnel exposure to infectious materials manipulated within the cabinet. Class II cabinets are designed so that horizontally nebulized spores introduced into the cabinet will be captured by the downward flowing cabinet air within fourteen inches⁸ of travel. Therefore, as a general rule of thumb, keeping clean materials at least one foot away from aerosol-generating activities will minimize the potential for cross-contamination.

The workflow should be from "clean to dirty" (Figure 11). Materials and supplies should be placed in the cabinet in such a way as to limit the movement of "dirty" items over "clean" ones.

Several measures can be taken to reduce the chance for cross-contamination of materials when working in a BSC. Opened tubes or bottles should not be held in a vertical position. Investigators working with Petri dishes and tissue culture plates should hold the lid above the open sterile surface to minimize direct impaction of downward air. Bottle or tube caps should not be placed on the towel. Items should be recapped or covered as soon as possible.

Open flames are not required in the near microbe-free environment of a biological safety cabinet. On an open bench, flaming the neck of a culture vessel will create an upward air current that prevents microorganisms from falling into the tube or flask. An open flame in a BSC, however, creates turbulence that disrupts the pattern of HEPA-filtered air being supplied to the work surface. When deemed absolutely necessary, touch-plate micro burners equipped with a pilot light to provide a flame on demand may be used. Internal cabinet air disturbance and heat buildup will be minimized. The burner must be turned off when work is completed. Small electric “furnaces” are available for decontaminating bacteriological loops and needles and are preferable to an open flame inside the BSC. Disposable or recyclable sterile loops should be used whenever possible.

Aspirator bottles or suction flasks should be connected to an overflow collection flask containing appropriate disinfectant, and to an in-line HEPA or equivalent filter (Figure 12). This combination will provide protection to the central building vacuum system or vacuum pump, as well as to the personnel who service this equipment. Inactivation of aspirated materials can be accomplished by placing sufficient chemical decontamination solution into the flask to inactivate the microorganisms as they are collected. Once inactivation occurs, liquid materials can be disposed of as noninfectious waste.

Investigators must determine the appropriate method of decontaminating materials that will be removed from the BSC at the conclusion of the work. When chemical means are appropriate, suitable liquid disinfectant should be placed into the discard pan before work begins. Items should be introduced into the pan with minimum splatter and allowed appropriate contact time as per manufacturer’s instructions. Alternatively, liquids can be autoclaved prior to disposal. Contaminated items should be placed into a biohazard bag, discard tray, or other suitable container prior to removal from the BSC.

When a steam autoclave is used, contaminated materials should be placed into a biohazard bag or discard pan containing enough water to ensure steam generation during the autoclave cycle. The bag should be taped shut or the discard pan should be covered in the BSC prior to transfer to the autoclave. The bag should be transported and autoclaved in a leak proof tray or pan. It is a prudent practice to decontaminate the exterior surface of bags and pans just prior to removal from the cabinet.

Decontamination

Cabinet Surface Decontamination

With the cabinet blower running, all containers and equipment should be surface decontaminated and removed from the cabinet when work is completed. At the end of the workday, the final surface decontamination of the cabinet should include a wipe-down of the work surface, the cabinet's sides and back and the interior of the glass. If necessary, the cabinet should also be monitored for radioactivity and decontaminated when necessary. Investigators should remove their gloves and gowns in a manner to prevent contamination of unprotected skin and aerosol generation and wash their hands as the final step in safe microbiological practices. The cabinet blower may be turned off after these operations are completed, or left on.

Small spills within the operating BSC can be handled immediately by removing the contaminated absorbent paper toweling and placing it into the biohazard bag or receptacle. Any splatter onto items within the cabinet, as well as the cabinet interior, should be immediately cleaned up with a towel dampened with an appropriate decontaminating solution. Gloves should be changed after the work surface is decontaminated and before placing clean absorbent toweling in the cabinet. Hands should be washed whenever gloves are changed or removed.

Spills large enough to result in liquids flowing through the front or rear grilles require decontamination that is more extensive. All items within the cabinet should be surface decontaminated and removed. After ensuring that the drain valve is closed, decontaminating solution can be poured onto the work surface and through the grille(s) into the drain pan.

Twenty to 30 minutes is generally considered an appropriate contact time for decontamination, but this varies with the disinfectant and the microbiological agent. Manufacturer's directions should be followed. The spilled fluid and disinfectant solution on the work surface should be absorbed with paper towels and discarded into a biohazard bag. The drain pan should be emptied into a collection vessel containing disinfectant. A hose barb and flexible tube should be attached to the drain valve and be of sufficient length to allow the open end to be submerged in the disinfectant within the collection vessel. This procedure serves to minimize aerosol generation. The drain pan should be flushed with water and the drain tube removed.

Should the spilled liquid contain radioactive material, a similar procedure can be followed. Radiation safety personnel should be contacted for specific instructions.

Periodic removal of the cabinetwork surface and/or grilles after the completion of drain pan decontamination may be justified because of dirty drain pan surfaces and grilles, which ultimately could occlude the drain valve or block airflow. However, extreme caution should be observed on wiping these surfaces to avoid injury

from broken glass that may be present and sharp metal edges. Always use disposable paper toweling and avoid applying harsh force. Wipe dirty surfaces gently. Never leave toweling on the drain pan because the paper could block the drain valve or the air passages in the cabinet.

Gas Decontamination

BSCs that have been used for work involving infectious materials must be decontaminated before HEPA filters are changed or internal repair work is done.²⁰⁻²³ Before a BSC is relocated, a risk assessment considering the agents manipulated within the BSC must be performed to determine the need and method for decontamination. The most common decontamination method uses formaldehyde gas, although more recently, hydrogen peroxide vapor²¹ and chlorine dioxide gas have been used successfully.

Section VI—Facility and Engineering Requirements

Secondary Barriers

Whereas BSCs are considered to be the primary containment barrier for manipulation of infectious materials, the laboratory room itself is considered to be the secondary containment barrier.²⁴ Inward directional airflow is established²⁵ by exhausting a greater volume of air than is supplied to a given laboratory and by drawing makeup air from the adjacent space. This is optional at BSL-2 but must be maintained at BSL-3 and BSL-4.^{1,26} The air balance for the entire facility should be established and maintained to ensure that airflow is from areas of least to greater potential contamination.

Building Exhaust

At BSL-3 and BSL-4, exhaust laboratory air must be directly exhausted to the outside since it is considered potentially contaminated. This concept is referred to as a dedicated, single-pass exhaust system. The exhausted room air can be HEPA-filtered when a high level of aerosol containment is needed, which is always true at BSL-4 and may be optional at BSL-3. When the building exhaust system is used to vent a ducted BSC, the system must have sufficient capacity to maintain the exhaust flow if changes in the static pressure within the system should occur. The connection to a BSC must be constant air volume (CAV).

The HVAC exhaust system must be sized to handle both the room exhaust and the exhaust requirements of all containment devices that may be present. Adequate supply air must be provided to ensure appropriate function of the exhaust system. Right angle bends changing duct diameters and transitional connections within the systems will add to the demand on the exhaust fan. The building exhaust air should be discharged away from supply air intakes, to prevent re-entrainment of laboratory exhaust air into the building air supply system. Refer to recognized design guides for locating the exhaust terminus relative to nearby air intakes.²⁷

Utility Services

Utility services needed within a BSC must be planned carefully. Protection of vacuum systems must be addressed (Figure 12). Electrical outlets inside the cabinet must be protected by ground fault circuit interrupters and should be supplied by an independent circuit. When propane or natural gas is provided, a clearly marked emergency gas shut-off valve outside the cabinet must be installed for fire safety. All non-electrical utility services should have exposed, accessible shut-off valves. The use of compressed air within a BSC must be carefully considered and controlled to prevent aerosol production and reduce the potential for vessel pressurization.

Ultraviolet Lamps

Ultraviolet (UV) lamps are not recommended in BSCs⁸ nor are they necessary. If installed, UV lamps must be cleaned weekly to remove any dust and dirt that may block the germicidal effectiveness of the ultraviolet light. The lamps should be checked weekly with a UV meter to ensure that the appropriate intensity of UV light is being emitted. UV lamps must be turned off when the room is occupied to protect eyes and skin from UV exposure, which can burn the cornea and cause skin cancer. If the cabinet has a sliding sash, close the sash when operating the UV lamp.

BSC Placement

BSCs were developed (see Section I) as workstations to provide personnel, environmental and product protection during the manipulation of infectious microorganisms. Certain considerations must be met to ensure maximum effectiveness of these primary barriers. Whenever possible, adequate clearance should be provided behind and on each side of the cabinet to allow easy access for maintenance and to ensure that the cabinet air re-circulated to the laboratory is not hindered. A 12 to 14 inch clearance above the cabinet may be required to provide for accurate air velocity measurement across the exhaust filter surface^{28,29} and for exhaust filter changes. When the BSC is hard-ducted or connected by a canopy unit to the ventilation system, adequate space must be provided so that the configuration of the ductwork will not interfere with airflow. The canopy unit must provide adequate access to the exhaust HEPA filter for testing.

The ideal location for the biological safety cabinet is remote from the entry (i.e., the rear of the laboratory away from traffic), since people walking parallel to the face of a BSC can disrupt the air curtain.^{16,20,30} The air curtain created at the front of the cabinet is quite fragile, amounting to a nominal inward and downward velocity of 1 mph. Open windows, air supply registers, portable fans or laboratory equipment that creates air movement (e.g., centrifuges, vacuum pumps) should not be located near the BSC. Similarly, chemical fume hoods must not be located close to BSCs.

HEPA Filters

HEPA filters, whether part of a building exhaust system or part of a cabinet, will require replacement when they become loaded to the extent that sufficient airflow can no longer be maintained. In most instances, filters must be decontaminated before removal. To contain the formaldehyde gas typically used for microbiological decontamination, exhaust systems containing HEPA filters require airtight dampers to be installed on both the inlet and discharge side of the filter housing. This ensures containment of the gas inside the filter housing during decontamination. Access panel ports in the filter housing also allow for performance testing of the HEPA filter. (See Section VII.)

A bag-in/bag-out filter assembly^{3,17} (Figure 13) can be used in situations where HEPA filtration is necessary for operations involving biohazardous materials and hazardous or toxic chemicals. The bag-in/bag-out system is used when it is not possible to gas or vapor decontaminate the HEPA filters, or when hazardous chemicals or radionuclides have been used in the BSC, and provides protection against exposure for the maintenance personnel and the environment. Note, however, that this requirement must be identified at the time of purchase and installation; a bag-in/bag-out assembly cannot be added to a cabinet after-the-fact without an extensive engineering evaluation.

Section VII — Certification of BSCs

Development of Containment Standards

The evolution of containment equipment for varied research and diagnostic applications created the need for consistency in construction and performance. Federal Standard 209^{a,32,33} was developed to establish classes of air cleanliness and methods for monitoring clean workstations and clean rooms where HEPA filters are used to control airborne particulates.

The first “standard” to be developed specifically for BSCs¹² served as a Federal procurement specification for the NIH Class II, Type 1 (now called Type A1) BSC, which had a fixed or hinged front window or a vertical sliding sash, vertical downward airflow and HEPA-filtered supply and exhaust air. This specification described design criteria and defined prototype tests for microbiological aerosol challenge, velocity profiles, and leak testing of the HEPA filters. A similar procurement specification was generated³¹ when the Class II, Type 2 (now called Type B1) BSC was developed.

a Federal Standard No. 209E9 has been replaced by ISO 14644. This standard does not apply to BSCs and should not be considered a basis for their performance or integrity certification. However, the methodology of ISO 14644 can be used to quantify the particle count within the work area of a BSC. ISO 14644 defines how to classify a clean room/clean zone. Performance tests and procedures needed to achieve a specified cleanliness classification are outlined by the Institute of Environmental Sciences and Technology’s IEST-RP-CC-006.

National Sanitation Foundation (NSF) Standard #49 for Class II BSCs was first published in 1976, providing the first independent standard for design, manufacture and testing of BSCs. This standard “replaced” the NIH specifications, which were being used by other institutions and organizations purchasing BSCs. NSF/ANSI Standard 49—2007^{b,8} incorporates current specifications regarding design, materials, construction, and testing. This Standard for BSCs establishes performance criteria and provides the minimum testing requirements that are accepted in the United States. Cabinets, which meet the Standard and are certified by NSF bear an NSF Mark.

NSF/ANSI Standard 49—2007 pertains to all models of Class II cabinets (Type A1, A2, B1, B2) and provides a series of specifications regarding:

- Design/construction
- Performance
- Installation recommendations
- Recommended microbiological decontamination procedure
- References and specifications pertinent to Class II Biosafety Cabinetry

Annex F of NSF/ANSI Standard 49—2007, which covers field-testing of BSCs, is now a normative part of the Standard. This Standard is reviewed periodically by a committee of experts to ensure that it remains consistent with developing technologies.

The operational integrity of a BSC must be validated before it is placed into service and after it has been repaired or relocated. Relocation may break the HEPA filter seals or otherwise damage the filters or the cabinet. Each BSC should be tested and certified at least annually to ensure continued, proper operation.

On-site field-testing (NSF/ANSI Standard 49—2007 Annex F plus Addendum #1) must be performed by experienced, qualified personnel. Some basic information is included in the Standard to assist in understanding the frequency and kinds of tests to be performed. In 1993, NSF began a program for accreditation of certifiers based on written and practical examinations. Education and training programs for persons seeking accreditation as qualified to perform all field certification tests are offered by a variety of organizations. Selecting competent individuals to perform testing and certification is important. It is suggested that the institutional BSO be consulted when identifying companies qualified to conduct the necessary field performance tests.

b The standard can be ordered from the NSF for a nominal fee at NSF International, 789 North Dixboro Road, P.O. Box 130140, Ann Arbor, Michigan, 48113-0140; Telephone: 734-769-8010; Fax: 734-769-0190; e-mail: info@nsf.org; Telex 753215 NSF INTL.

It is strongly recommended that, whenever possible, accredited field certifiers are used to test and certify BSCs. If in-house personnel are performing the certifications, then these individuals should become accredited.

The annual tests applicable to each of the three classes of BSCs are listed in Table 3. Table 4 indicates where to find information regarding the conduct of selected tests. BSCs consistently perform well when proper annual certification procedures are followed; cabinet or filter failures tend to occur infrequently.

Performance Testing BSCs in the Field

Class II BSCs are the primary containment devices that protect the worker, product and environment from exposure to microbiological agents. BSC operation, as specified by NSF/ANSI Standard 49—2007, Annex F plus Addendum #1 needs to be verified at the time of installation and, as a minimum, annually thereafter. The purpose and acceptance level of the operational tests (Table 3) ensure the balance of inflow and exhaust air, the distribution of air onto the work surface, and the integrity of the cabinet and the filters. Other tests check electrical and physical features of the BSC.

- A. *Down flow Velocity Profile Test:* This test is performed to measure the velocity of air moving through the cabinet workspace, and is to be performed on all Class II BSCs.
- B. *Inflow Velocity Test:* This test is performed to determine the calculated or directly measured velocity through the work access opening, to verify the nominal set point average inflow velocity and to calculate the exhaust airflow volume rate.
- C. *Airflow Smoke Patterns Test:* This test is performed to determine if:
1) the airflow along the entire perimeter of the work access opening is inward; 2) if airflow within the work area is downward with no dead spots or refluxing; 3) if ambient air passes onto or over the work surface; and 4) if there is no escape to the outside of the cabinet at the sides and top of the window. The smoke test is an indicator of airflow direction, not velocity.
- D. *HEPA Filter Leak Test:* This test is performed to determine the integrity of supply and exhaust HEPA filters, filter housing and filter mounting frames while the cabinet is operated at the nominal set point velocities. An aerosol in the form of generated particulates of dioctylphthalate (DOP) or an accepted alternative (e.g., poly alpha olefin (PAO), di(2-ethylhexyl) sebecate, polyethylene glycol and medical grade light mineral oil) is required for leak-testing HEPA filters and their seals. The aerosol is generated on the intake side of the filter and particles passing through the filter or around the seal are measured with a photometer on the discharge side. This test is suitable for ascertaining the integrity of all HEPA filters.

- E. *Cabinet Integrity Test (A1 Cabinets only)*: This pressure holding test is performed to determine if exterior surfaces of all plenums, welds, gaskets and plenum penetrations or seals are free of leaks. In the field, it need only be performed on Type A1 cabinets at the time of initial installation when the BSC is in a free-standing position (all four sides are easily accessible) in the room in which it will be used, after a cabinet has been relocated to a new location, and again after removal of access panels to plenums for repairs or a filter change. This test may also be performed on fully installed cabinets. Cabinet integrity can also be checked using the bubble test; liquid soap can be spread along welds, gaskets and penetrations to visualize air leaks that may occur.
- F. *Electrical Leakage and Ground Circuit Resistance and Polarity Tests*: Electrical testing has been taken out of NSF/ANSI 49 Standard—2007 for new cabinets certified under the this Standard. This responsibility has been turned over to UL. All new cabinets must meet UL 61010A-1 in order to be certified by NSF. These safety tests are performed to determine if a potential shock hazard exists by measuring the electrical leakage, polarity, ground fault interrupter function and ground circuit resistance to the cabinet connection. An electrical technician other than the field certification personnel may perform the tests at the same time the other field certification tests are conducted. The polarity of electrical outlets is checked (Table 3, E). The ground fault circuit interrupter should trip when approximately five milliamperes (mA) is applied.
- G. *Lighting Intensity Test*: This test is performed to measure the light intensity on the work surface of the cabinet as an aid in minimizing cabinet operator fatigue.
- H. *Vibration Test*: This test is performed to determine the amount of vibration in an operating cabinet as a guide to satisfactory mechanical performance, as an aid in minimizing cabinet operator fatigue and to prevent damage to delicate tissue culture specimens.
- I. *Noise Level Test*: This test is performed to measure the noise levels produced by the cabinets, as a guide to satisfactory mechanical performance and an aid in minimizing cabinet operator fatigue.
- J. *UV Lamp Test*: A few BSCs have UV lamps. When used, they must be tested periodically to ensure that their energy output is sufficient to kill microorganisms. The surface on the bulb should be cleaned with 70% ethanol prior to performing this test. Five minutes after the lamp has been turned on, the sensor of the UV meter is placed in the center of the work surface. The radiation output should not be less than 40 microwatts per square centimeter at a wavelength of 254 nanometers (nm).

Finally, accurate test results can only be assured when the testing equipment is properly maintained and calibrated. It is appropriate to request the calibration information for the test equipment being used by the certifier.

Table 1. Selection of a Safety Cabinet through Risk Assessment

Biological Risk Assessed	Protection Provided			BSC Class
	Personnel	Product	Environmental	
BSL 1 – 3	Yes	No	Yes	I
BSL 1 – 3	Yes	Yes	Yes	II (A1, A2, B1, B2)
BSL – 4	Yes	Yes	Yes	III; II—When used in suit room with suit

Table 2. Comparison of Biosafety Cabinet Characteristics

BSC Class	Face Velocity	Airflow Pattern	Applications	
			Nonvolatile Toxic Chemicals and Radionuclides	Volatile Toxic Chemicals and Radionuclides
I	75	In at front through HEPA to the outside or into the room through HEPA (Figure 2)	Yes	When exhausted outdoors ^{1,2}
II, A1	75	70% recirculated to the cabinet work area through HEPA; 30% balance can be exhausted through HEPA back into the room or to outside through a canopy unit (Figure 3)	Yes (minute amounts)	No
II, B1	100	30% recirculated, 70% exhausted. Exhaust cabinet air must pass through a dedicated duct to the outside through a HEPA filter (Figures 5A, 5B)	Yes	Yes (minute amounts) ^{1,2}
I, B2	100	No recirculation; total exhaust to the outside through a HEPA filter (Figure 6)	Yes	Yes (small amounts) ^{1,2}
II, A2	100	Similar to II, A1, but has 100 lfm intake air velocity and plenums are under negative pressure to room; exhaust air can be ducted to the outside through a canopy unit (Figure 7)	Yes	When exhausted outdoors (FORMALLY “B3”) (minute amounts) ^{1,2}

BSC Class	Face Velocity	Airflow Pattern	Applications	
			Nonvolatile Toxic Chemicals and Radionuclides	Volatile Toxic Chemicals and Radionuclides
III	N/A	Supply air is HEPA filtered. Exhaust air passes through two HEPA filters in series and is exhausted to the outside via a hard connection (Figure 8)	Yes	Yes (small amounts) ^{1,2}

¹ Installation requires a special duct to the outside, an in-line charcoal filter, and a spark proof (explosion proof) motor and other electrical components in the cabinet. Discharge of a Class I or Class II, Type A2 cabinet into a room should not occur if volatile chemicals are used.

² In no instance should the chemical concentration approach the lower explosion limits of the compounds.

Table 3. Field Performance Tests Applied to the Three Classes of Biological Safety Cabinets

Test Performed for	Biosafety Cabinet		
	Class I	Class II	Class III
Primary Containment			
Cabinet Integrity	N/A	A (A1 Only)	A
HEPA Filter Leak	Required	Required	Required
Down flow Velocity	N/A	Required	N/A
Face Velocity	Required	Required	N/A
Negative Pressure / Ventilation Rate	B	N/A	Required
Airflow Smoke Patterns	Required	Required	E, F
Alarms and Interlocks	C, D	C, D	Required
Electrical Safety			
Electrical Leakage, etc.	E, D	E, D	E, D
Ground Fault Interrupter	D	D	D

Test Performed for	Biosafety Cabinet		
	Class I	Class II	Class III
Other			
Lighting Intensity	E	E	E
UV Intensity	C, E	C, E	C, E
Noise Level	E	E	E
Vibration	E	E	E

Required Required during certification.

- A Required for proper certification if the cabinet is new, has been moved or panels have been removed for maintenance.
- B If used with gloves.
- C If present.
- D Encouraged for electrical safety.
- E Optional, at the discretion of the user.
- F Used to determine air distribution within cabinet for clean to dirty procedures.
- N/A Not applicable.

Table 4. Reference for Applicable Containment Test

Test	Biosafety Cabinet Type		
	Class I	Class II	Class III
HEPA Filter Leak	(F.5) ¹	(F.5)	(F.5)
Airflow Smoke Pattern	No smoke shall reflux out of BSC once drawn in	(F.4)	N/A
Cabinet Integrity	N/A	(F.6)	[p.138 – 141] ²
Face Velocity Open Front	[75-125 lfm]	75 lfm—type A1; 100 lfm type A2, B1 & B2: (F.3)	N/A
Face VelocityGloves Ports / No Gloves	150 lfm	N/A	N/A
Water Gauge Pressure Glove Ports and Gloves	N/A	N/A	(-0.5 "w.c.") [p. 145]
Down flow Velocity	N/A	(F.2)	N/A

¹ Parenthetical references are to the NSF/ANSI Standard 49—2007; letters and numerals indicate specific sections and subsections.

² Bracketed reference [] is to the Laboratory Safety Monograph; page numbers are indicated.

Figure 1. HEPA filters are typically constructed of paper-thin sheets of borosilicate medium, pleated to increase surface area, and affixed to a frame. Aluminum separators are often added for stability.

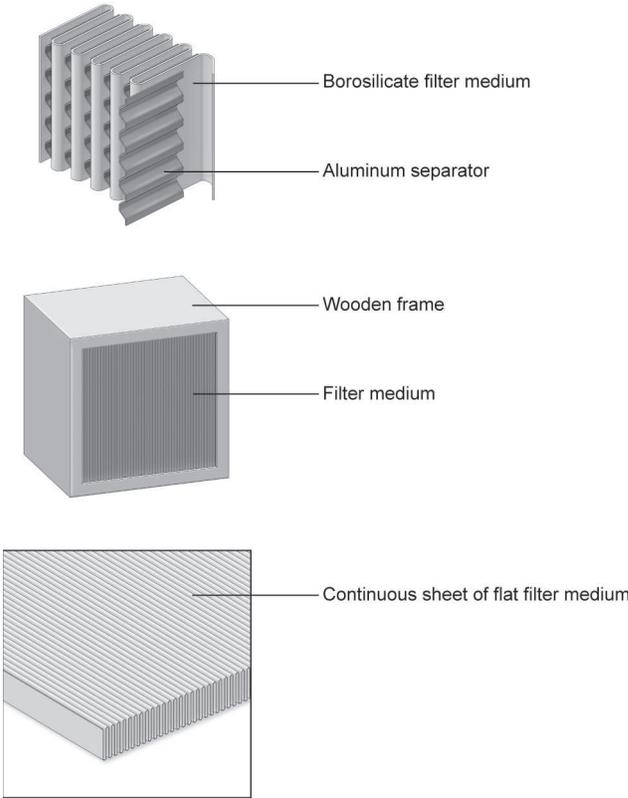


Figure 2. The Class I BSC (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) exhaust plenum. *Note:* The cabinet needs to be hard connected to the building exhaust system if toxic vapors are to be used.

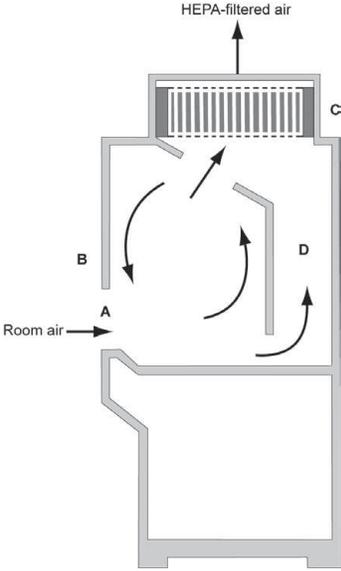


Figure 3. The Class II, Type A1 BSC (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) common plenum; (F) blower.

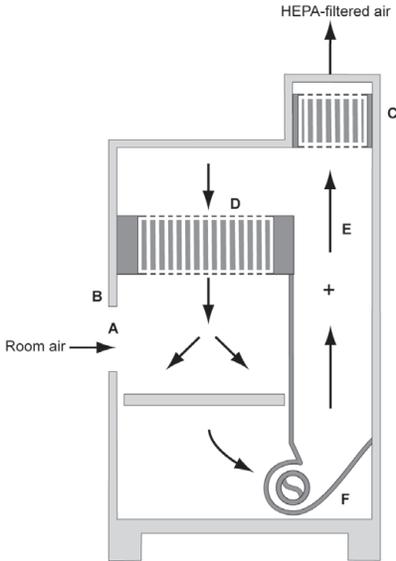


Figure 4. Canopy (thimble) unit for ducting a Class II, Type A BSC (A) balancing damper; (B) flexible connector to exhaust system; (C) cabinet exhaust HEPA filter housing; (D) canopy unit; (E) BSC. *Note:* There is a 1" gap between the canopy unit (D) and the exhaust filter housing (C), through which room air is exhausted.

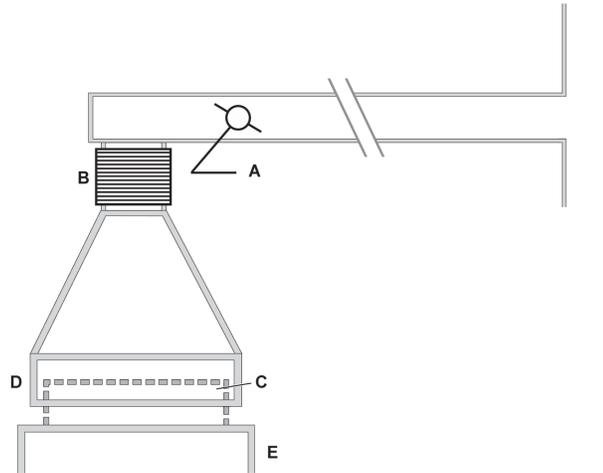


Figure 5A. The Class II, Type B1 BSC (classic design) (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) negative pressure dedicated exhaust plenum; (F) blower; (G) additional HEPA filter for supply air. *Note:* The cabinet exhaust needs to be hard connected to the building exhaust system.

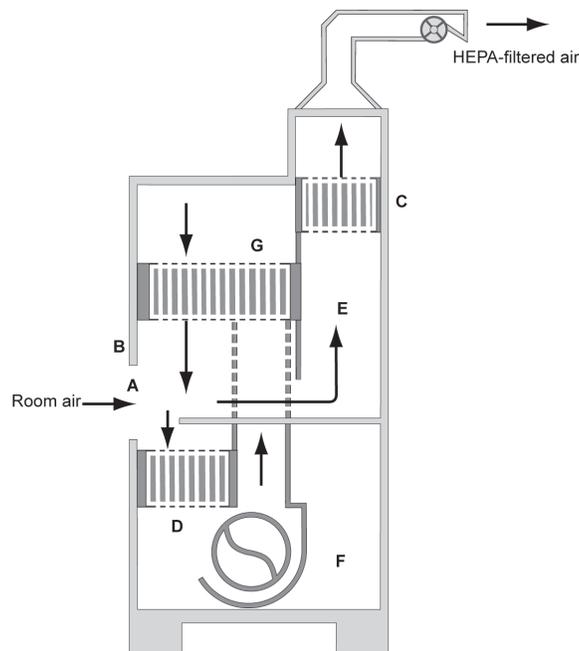


Figure 5B. The Class II, Type B1 BSC (bench top design) (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply plenum; (E) supply HEPA filter; (F) blower; (G) negative pressure exhaust plenum. *Note:* The cabinet exhaust needs to be hard connected to the building exhaust system.

Connection to the building exhaust system is required.

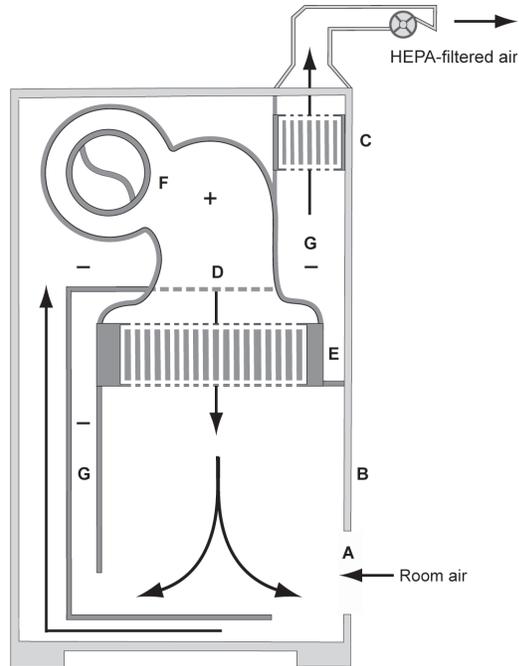


Figure 6. The Class II, Type B2 BSC (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) negative pressure exhaust plenum. *Note:* The carbon filter in the exhaust system is not shown. The cabinet needs to be hard connected to the building exhaust system.

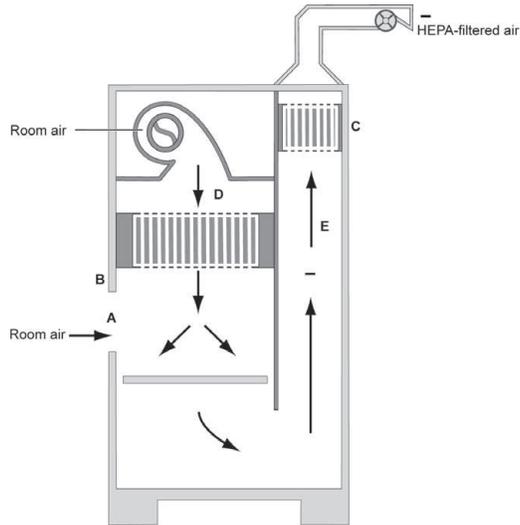


Figure 7. The tabletop model of a Class II, Type A2 BSC (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) positive pressure common plenum; (F) negative pressure plenum. The Class II Type A2 BSC is not equivalent to what was formerly called a Class II Type B3 unless it is connected to the laboratory exhaust system. *Note:* The A2 BSC should be canopy connected to the exhaust system.

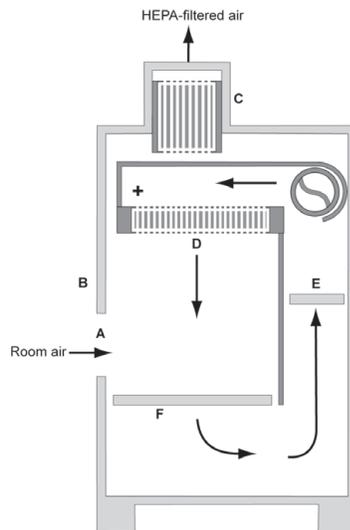


Figure 8. The Class III BSC (A) glove ports with O-ring for attaching arm-length gloves to cabinet; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) double-ended autoclave or pass-through box. *Note:* A chemical dunk tank may be installed which would be located beneath the work surface of the BSC with access from above. The cabinet exhaust needs to be hard connected to an exhaust system where the fan is generally separate from the exhaust fans of the facility ventilation system. The exhaust air must be double HEPA-filtered or HEPA-filtered and incinerated.

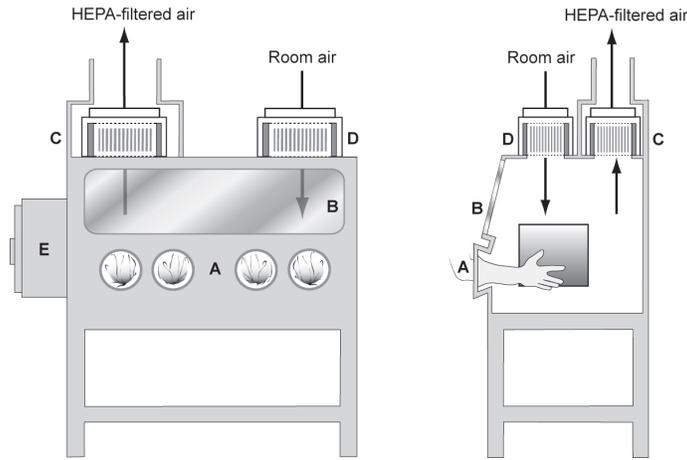


Figure 9A. The horizontal laminar flow “clean bench” (A) front opening; (B) supply grille; (C) supply HEPA filter; (D) supply plenum; (E) blower.

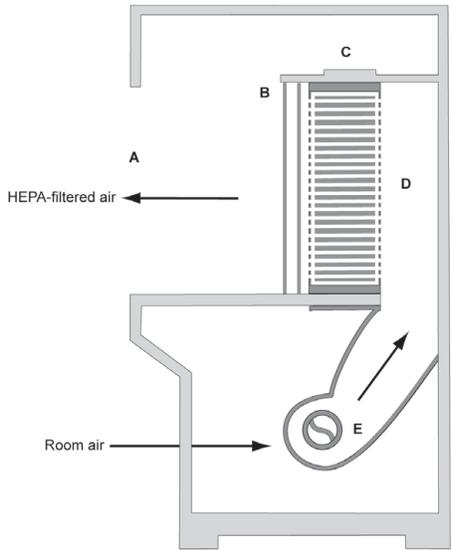


Figure 9B. The vertical laminar flow “clean bench” (A) front opening; (B) sash; (C) supply HEPA filter; (D) blower. *Note:* Some vertical flow clean benches have recirculated air through front and/or rear perforated grilles.

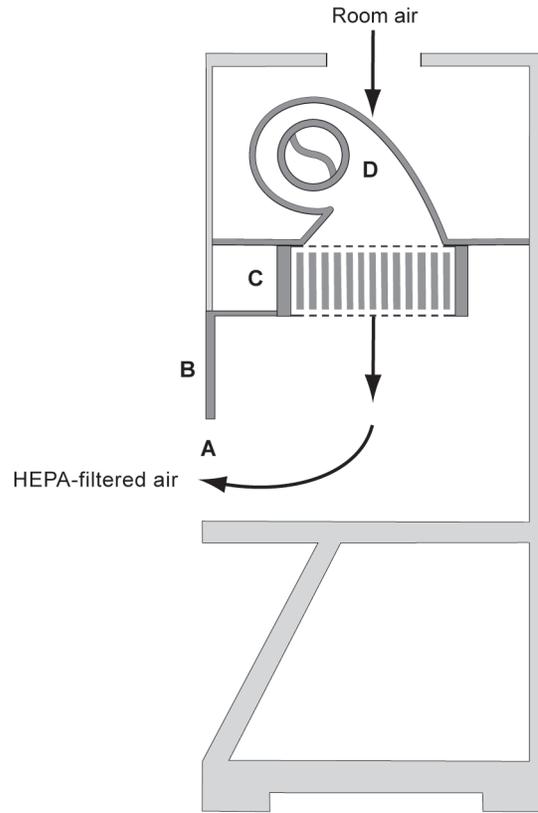


Figure 10. A modified containment cabinet or Class I BSC can be used for labeling infectious microorganisms with I^{125} . (A) arm holes; (B) Lexan[®] hinged doors; (C) exhaust charcoal filter; (D) exhaust HEPA filter; (E) filter housing with required connection to building exhaust (see also Figure 13).

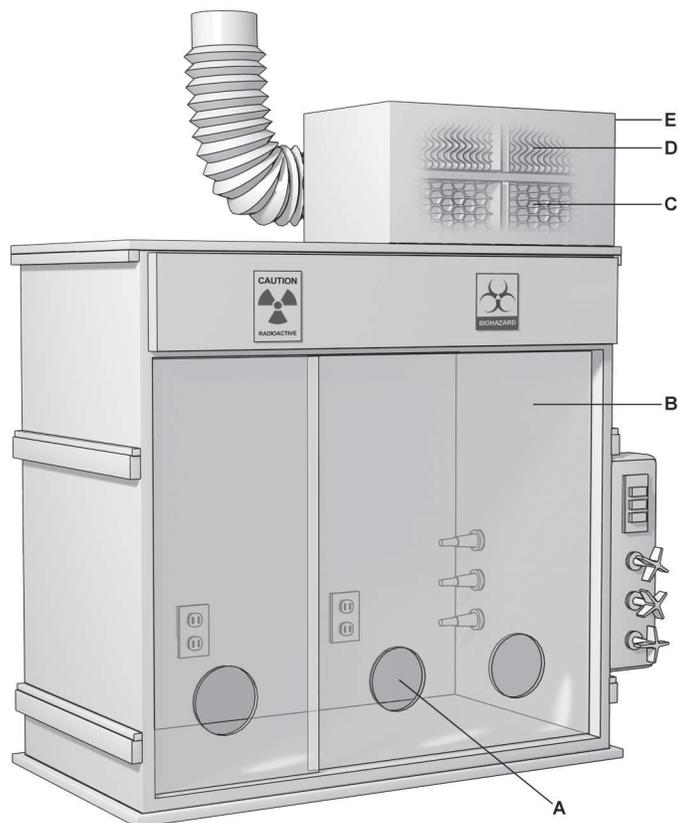


Figure 11. A typical layout for working “clean to dirty” within a Class II BSC. Clean cultures (left) can be inoculated (center); contaminated pipettes can be discarded in the shallow pan and other contaminated materials can be placed in the biohazard bag (right). This arrangement is reversed for left-handed persons.

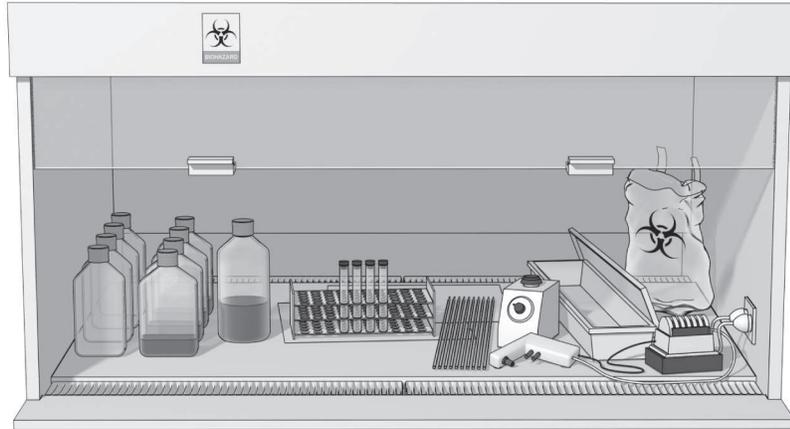


Figure 12. One method to protect a house vacuum system during aspiration of infectious fluids. The left suction flask (A) is used to collect the contaminated fluids into a suitable decontamination solution; the right flask (B) serves as a fluid overflow collection vessel. An in-line HEPA filter (C) is used to protect the vacuum system (D) from aerosolized microorganisms.

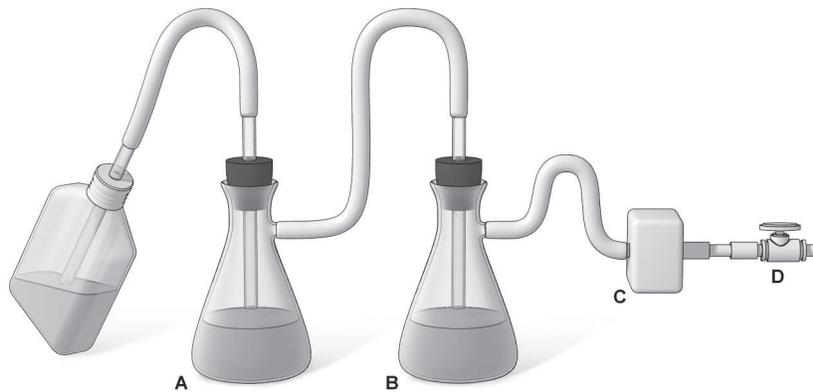
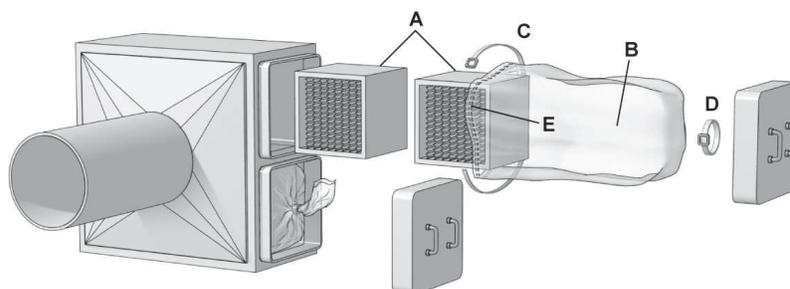


Figure 13. A bag-in-bag-out filter enclosure allows for the removal of the contaminated filter without worker exposure. (A) filters; (B) bags; (C) safety straps; (D) cinching straps; (E) shock cord located in the mouth of the PVC bag restricts the bag around the second rib of the housing lip.



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Appendix B—Decontamination and Disinfection

This section describes basic strategies for decontaminating surfaces, items, and areas in laboratories to eliminate the possibility of transmission of infectious agents to laboratory workers, the general public, and the environment. Factors necessary for environmentally mediated infection transmission are reviewed as well as methods for sterilization and disinfection and the levels of antimicrobial activity associated with liquid chemical germicides. General approaches are emphasized, not detailed protocols and methods. The principles of sterilization and disinfection are stated and compared.

Environmentally Mediated Infection Transmission

Environmentally associated laboratory infections can be transmitted directly or indirectly from environmental sources (e.g., air, contaminated fomites and laboratory instruments, and aerosols) to laboratory staff. Fortunately, LAI are rare events¹ because there are a number of requirements necessary for environmental transmission to occur.² Commonly referred to as the “chain of infection” they include: presence of a pathogen of sufficient virulence, relatively high concentration of the pathogen (i.e., infectious dose), and a mechanism of transmission of the pathogen from environment to the host, a correct portal of entry to a susceptible host.

To accomplish successful transmission from an environmental source, all of these requirements for the “chain of infection” must be present. The absence of any one element will prevent transmission. Additionally, the pathogen in question must overcome environmental stresses to retain viability, virulence, and the capability to initiate infection in the host. In the laboratory setting, high concentrations of pathogens can be common. Reduction of environmental microbial contamination by conventional cleaning procedures is often enough to prevent environmentally mediated transmission. However, it is the general practice in laboratories to use sterilization methods to remove the potential for infection transmission.

Principles of Sterilization and Disinfection

In order to implement a laboratory biosafety program it is important to understand the principles of decontamination, cleaning, sterilization, and disinfection. We review here the definitions of sterilization, disinfection, antisepsis, decontamination, and sanitization to avoid misuse and confusion. The definitions and implied capabilities of each inactivation procedure are discussed with an emphasis on achievement and in some cases, monitoring of each state.

Sterilization

Any item, device, or solution is considered to be sterile when it is completely free of all living microorganisms and viruses. The definition is categorical and absolute (i.e., an item is either sterile or it is not). A sterilization *procedure* is one that kills all microorganisms, including high numbers of bacterial endospores. Sterilization can be accomplished by heat, ethylene oxide gas, hydrogen peroxide gas, plasma, ozone, and radiation (in industry). From an operational standpoint, a sterilization procedure cannot be categorically defined. Rather, the procedure is defined as a process, after which the probability of a microorganism surviving on an item subjected to treatment is less than one in one million (10⁻⁶). This is referred to as the “sterility assurance level.”^{3,4}

Disinfection

Disinfection is generally a less lethal process than sterilization. It eliminates nearly all recognized pathogenic microorganisms but not necessarily all microbial forms (e.g., bacterial spores) on inanimate objects. Disinfection does not ensure an “overkill” and therefore lacks the margin of safety achieved by sterilization procedures. The effectiveness of a disinfection procedure is controlled significantly by a number of factors, each one of which may have a pronounced effect on the end result. Among these are:

- the nature and number of contaminating microorganisms (especially the presence of bacterial spores);
- the amount of organic matter present (e.g., soil, feces, and blood);
- the type and condition of instruments, devices, and materials to be disinfected;
- the temperature.

Disinfection is a procedure that reduces the level of microbial contamination, but there is a broad range of activity that extends from sterility at one extreme to a minimal reduction in the number of microbial contaminants at the other. By definition, chemical disinfection and in particular, high-level disinfection differs from chemical sterilization by its lack of sporicidal power. This is an over simplification of the actual situation because a few chemical germicides used as disinfectants do, in fact, kill large numbers of spores even though high concentrations and several hours of exposure may be required. Non-sporicidal disinfectants may differ in their capacity to accomplish disinfection or decontamination. Some germicides rapidly kill only the ordinary vegetative forms of bacteria such as staphylococci and streptococci, some forms of fungi, and lipid-containing viruses, whereas others are effective against such relatively resistant organisms as *Mycobacterium tuberculosis* var. *bovis*, non-lipid viruses, and most forms of fungi.

Spaulding Classification

In 1972, Dr. Earl Spaulding⁵ proposed a system for classifying liquid chemical germicides and inanimate surfaces that has been used subsequently by CDC, FDA, and opinion leaders in the United States. This system, as it applies to device surfaces, is divided into three general categories based on the theoretical risk of infection if the surfaces are contaminated at time of use. From the laboratory perspective, these categories are:

- critical—instruments or devices that are exposed to normally sterile areas of the body require sterilization;
- semi-critical—instruments or devices that touch mucous membranes may be either sterilized or disinfected;
- non-critical—instruments or devices that touch skin or come into contact with persons only indirectly can be either cleaned and then disinfected with an intermediate-level disinfectant, sanitized with a low-level disinfectant, or simply cleaned with soap and water.

In 1991, microbiologists at CDC proposed an additional category, environmental surfaces (e.g., floors, walls, and other “housekeeping surfaces”) that do not make direct contact with a person’s skin.⁶ Spaulding also classified chemical germicides by activity level:

High-level Disinfection

This procedure kills vegetative microorganisms and inactivates viruses, but not necessarily high numbers of bacterial spores. Such disinfectants are capable of sterilization when the contact time is relatively long (e.g., 6 to 10 hours). As high-level disinfectants, they are used for relatively short periods of time (e.g., 10 to 30 minutes). These chemical germicides are potent sporicides and, in the United States, are classified by the FDA as sterilant/disinfectants. They are formulated for use on medical devices, but not on environmental surfaces such as laboratory benches or floors.⁷

Intermediate-level Disinfection

This procedure kills vegetative microorganisms, including *Mycobacterium tuberculosis*, all fungi, and inactivates most viruses. Chemical germicides used in this procedure often correspond to Environmental Protection Agency (EPA)-approved “hospital disinfectants” that are also “tuberculocidal.” They are used commonly in laboratories for disinfection of laboratory benches and as part of detergent germicides used for housekeeping purposes.

Low-level Disinfection

This procedure kills most vegetative bacteria except *M. tuberculosis*, some fungi, and inactivates some viruses. The EPA approves chemical germicides used in this procedure in the US as “hospital disinfectants” or “sanitizers.”

Decontamination in the Microbiology Laboratory

Decontamination in the microbiology laboratory must be carried out with great care. In this arena, decontamination may entail disinfection of work surfaces, decontamination of equipment so it is safe to handle, or may require sterilization. Regardless of the method, the purpose of decontamination is to protect the laboratory worker, the environment, and anyone who enters the laboratory or handles laboratory products away from the laboratory. Reduction of cross-contamination in the laboratory is an added benefit.

Decontamination and Cleaning

Decontamination renders an area, device, item, or material safe to handle (i.e., safe in the context of being reasonably free from a risk of disease transmission). The primary objective is to reduce the level of microbial contamination so that infection transmission is eliminated. The decontamination process may be ordinary soap and water cleaning of an instrument, device, or area. In laboratory settings, decontamination of items, spent laboratory materials, and regulated laboratory wastes is often accomplished by a sterilization procedure such as steam autoclaving, perhaps the most cost-effective way of decontaminating a device or an item.

The presence of any organic matter necessitates longer contact time with a decontamination method if the item or area is not pre-cleaned. For example, a steam cycle used to sterilize pre-cleaned items is 20 minutes at 121°C. When steam sterilization is used to decontaminate items that have a high bio-burden and there is no pre-cleaning (i.e., infectious waste) the cycle is longer. Decontamination in laboratory settings often requires longer exposure times because pathogenic microorganisms may be protected from contact with the decontaminating agents.

Table 1. Descending Order of Resistance to Germicidal Chemicals

Bacterial Spores <i>Bacillus subtilis</i> , <i>Clostridium sporogenes</i>
▼
Mycobacteria <i>Mycobacterium tuberculosis</i> var. <i>bovis</i> , Nontuberculous mycobacteria
▼
Nonlipid or Small Viruses Poliovirus, Coxsackievirus, Rhinovirus
▼
Fungi <i>Trichophyton</i> spp., <i>Cryptococcus</i> spp., <i>Candida</i> spp.
▼
Vegetative Bacteria <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i> , <i>Salmonella choleraesuis</i> , Enterococci
▼
Lipid or Medium-size Viruses Herpes simplex virus, CMV, Respiratory syncytial virus, HBV, HCV, HIV, Hantavirus, Ebola virus

Note: There are exceptions to this list. *Pseudomonas* spp are sensitive to high-level disinfectants, but if they grow in water and form biofilms on surfaces, the protected cells can approach the resistance of bacterial spores to the same disinfectant. The same is true for the resistance to glutaraldehyde by some nontuberculous mycobacteria, some fungal ascospores of *Microascus cinereus* and *Cheatomium globosum*, and the pink pigmented *Methylobacteria*. Prions are also resistant to most liquid chemical germicides and are discussed in the last part of this section.

Chemical germicides used for decontamination range in activity from high-level disinfectants (i.e., high concentrations of sodium hypochlorite [chlorine bleach]), which might be used to decontaminate spills of cultured or concentrated infectious agents in research or clinical laboratories, to low-level disinfectants or sanitizers for general housekeeping purposes or spot decontamination of environmental surfaces in healthcare settings. Resistance of selected organisms to decontamination is presented in descending order in Table 1. If dangerous and highly infectious agents are present in a laboratory, the methods for decontamination of spills, laboratory equipment, BSC, or infectious waste are very significant and may include prolonged autoclave cycles, incineration or gaseous treatment of surfaces.

Decontamination of Large Spaces

Space decontamination is a specialized activity and should be performed by specialists with proper training and protective equipment.⁸ Decontamination requirements for BSL-3 and BSL-4 laboratory space have an impact on the design of these facilities. The interior surfaces of BSL-3 laboratories must be water resistant in order for them to be easily cleaned and decontaminated. Penetrations in these surfaces should be sealed or capable of being sealed for decontamination purposes. Thus, in the BSL-3 laboratory, surface decontamination, not fumigation, is the primary means of decontaminating space. Care should be taken that penetrations in the walls, floors and ceilings are kept to a minimum and are "sight sealed." Verification of the seals is usually not required for most BSL-3 laboratories. The BSL-4 laboratory design requires interior surfaces that are water resistant AND sealed to facilitate fumigation. These seals must be tested and verified to ensure containment in order to permit both liquid disinfection and fumigation. Periodic fumigation is required in the BSL-4 suit laboratory to allow routine maintenance and certification of equipment. Procedures for decontamination of large spaces such as incubators or rooms are varied and influenced significantly by the type of etiologic agent involved, the characteristics of the structure containing the space, and the materials present in the space. The primary methods for space decontamination follow.

Formaldehyde—Paraformaldehyde

Formaldehyde gas at a concentration of 0.3 grams/cubic foot for four hours is often used for space decontamination. Gaseous formaldehyde can be generated by heating flake paraformaldehyde (0.3 grams per cubic foot) in a frying pan, thereby converting it to formaldehyde gas. The humidity must be controlled and the system works optimally at 80% relative humidity. This method is effective in killing microorganisms but toxicity issues are present.^{1,9} Additional information on environmental and safety issues related to paraformaldehyde is available from the EPA Web site: www.epa.gov/pesticides.

Hydrogen Peroxide Vapor

Hydrogen peroxide can be vaporized and used for the decontamination of glove boxes as well as small room areas. Vapor phase hydrogen peroxide has been shown to be an effective sporicide at concentrations ranging from 0.5 mg/L to <10 mg/L. The optimal concentration of this agent is about 2.4 mg/L with a contact time of at least one hour. This system can be used to decontaminate glove boxes, walk in incubators and small rooms. An advantage of this system is that the end products (i.e., water) are not toxic. Low relative humidity can be used.¹⁰⁻¹⁴

Chlorine Dioxide Gas

Chlorine dioxide gas sterilization can be used for decontamination of laboratory rooms, equipment, glove boxes, and incubators. The concentration of gas at the

site of decontamination should be approximately 10 mg/L with contact time of one to two hours.

Chlorine dioxide possesses the bactericidal, virucidal and sporicidal properties of chlorine, but unlike chlorine, does not lead to the formation of trihalomethanes or combine with ammonia to form chlorinated organic products (chloramines). The gas cannot be compressed and stored in high-pressure cylinders, but is generated upon demand using a column-based solid phase generation system. Gas is diluted to the use concentration, usually between 10 and 30 mg/L. Within reasonable limits, a chlorine dioxide gas generation system is unaffected by the size or location of the ultimate destination for the gas. Relative humidity does need to be controlled and high humidities are optimal. Although most often used in closed sterilizers, the destination enclosure for the chlorine dioxide gas does not, in fact, need to be such a chamber. Because chlorine dioxide gas exits the generator at a modest positive pressure and flow rate, the enclosure also need not be evacuated and could be a sterility-testing isolator, a glove box or sealed BSC, or even a small room that could be sealed to prevent gas egress.¹⁵ Chlorine dioxide gas is rapidly broken down by light; care must be taken to eliminate light sources in spaces to be decontaminated.

Decontamination of Surfaces

Liquid chemical germicides formulated as disinfectants may be used for decontamination of large areas. The usual procedure is to flood the area with a disinfectant for periods up to several hours. This approach is messy and with some of the disinfectants used represents a toxic hazard to laboratory staff. For example, most of the “high-level” disinfectants on the United States market are formulated to use on instruments and medical devices and not on environmental surfaces. Intermediate and low-level disinfectants are formulated to use on fomites and environmental surfaces but lack the potency of a high-level disinfectant. For the most part intermediate and low level disinfectants can be safely used and, as with all disinfectants, the manufacturer’s instructions should be closely followed.⁷ Disinfectants that have been used for decontamination include sodium hypochlorite solutions at concentrations of 500 to 6000 parts per million (ppm), oxidative disinfectants such as hydrogen peroxide and peracetic acid, phenols, and iodophors.

Concentrations and exposure times vary depending on the formulation and the manufacturer’s instructions for use.^{6,16} See Table 2 for a list of chemical germicides and their activity levels. A spill control plan must be available in the laboratory. This plan should include the rationale for selection of the disinfecting agent, the approach to its application, contact time and other parameters. Agents requiring BSL-3 and BSL-4 containment pose a high risk to workers and possibly to the environment and should be managed by well-informed professional staff trained and equipped to work with concentrated material.

Table 2. Activity Levels of Selected Liquid Germicides^a

Procedure / Product	Aqueous Concentration	Activity Level
Sterilization		
glutaraldehyde	variable	
hydrogen peroxide	6 – 30%	
formaldehyde	6 – 8%	
chlorine dioxide	variable	
peracetic acid		
Disinfection		
glutaraldehyde	variable	high to intermediate
ortho-phthalaldehyde	0.5%	high
hydrogen peroxide	3 – 6%	high to intermediate
formaldehyde ^b	1 – 8%	high to low
chlorine dioxide	variable	high
peracetic acid	variable	high
chlorine compounds ^c	500 to 5000 mg/L free/available chlorine	Intermediate
alcohols (ethyl, isopropyl) ^d	70%	Intermediate
phenolic compounds	0.5 to 3%	intermediate to low
iodophor compounds ^e	30 – 50 mg/L free iodine up to 10,000 mg/L available iodine 0.1 – 0.2%	intermediate to low
quaternary ammonium compounds		low

- ^a This list of chemical germicides centers on generic formulations. A large number of commercial products based on these generic components can be considered for use. Users should ensure that commercial formulations are registered with EPA or by the FDA.
- ^b Because of the ongoing controversy of the role of formaldehyde as a potential occupational carcinogen, the use of formaldehyde is limited to certain specific circumstances under carefully controlled conditions (e.g., for the disinfection of certain hemodialysis equipment). There are no FDA cleared liquid chemical sterilant/disinfectants that contain formaldehyde.
- ^c Generic disinfectants containing chlorine are available in liquid or solid form (e.g., sodium or calcium hypochlorite). Although the indicated concentrations are rapid acting and broad-spectrum (tuberculocidal, bactericidal, fungicidal, and virucidal), no proprietary hypochlorite formulations are formally registered with EPA or cleared by FDA. Common household bleach is an excellent and inexpensive source of sodium hypochlorite. Concentrations between 500 and 1000 mg/L chlorine are appropriate for the vast majority of uses requiring an intermediate level of germicidal activity; higher concentrations are extremely corrosive as well as irritating to personnel, and their use should be limited to situations where there is an excessive amount of organic material or unusually high concentrations of microorganisms (e.g., spills of cultured material in the laboratory).

- ^d The effectiveness of alcohols as intermediate level germicides is limited because they evaporate rapidly, resulting in short contact times, and also lack the ability to penetrate residual organic material. They are rapidly tuberculocidal, bactericidal and fungicidal, but may vary in spectrum of virucidal activity (see text). Items to be disinfected with alcohols should be carefully pre-cleaned then totally submerged for an appropriate exposure time (e.g., 10 minutes).
- ^e Only those iodophors registered with EPA as hard-surface disinfectants should be used, closely following the manufacturer's instructions regarding proper dilution and product stability. Antiseptic iodophors are not suitable to disinfect devices, environmental surfaces, or medical instruments.

Special Infectious Agent Issues

Transmissible Spongiform Encephalopathy Agents (Prions)

The major exception to the rule in the previous discussion of microbial inactivation and decontamination is the causative agent of CJD or other prion agents responsible for transmissible spongiform encephalopathies of the central nervous system in humans or animals. Studies show that prions are resistant to conventional uses of heat and/or chemical germicides for the sterilization of instruments and devices. (See Section IX.)

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Appendix C—Transportation of Infectious Substances

An infectious substance is a material known to contain or reasonably expected to contain a pathogen. A pathogen is a microorganism (including bacteria, viruses, rickettsiae, parasites, fungi) or other agent, such as a proteinaceous infectious particle (prion), that can cause disease in humans or animals. Infectious substances may exist as purified and concentrated cultures, but may also be present in a variety of materials, such as body fluids or tissues. Transportation of infectious substances and materials that are known or suspected to contain them are regulated as hazardous materials by the United State Department of Transportation (DOT), foreign governments, and the International Civil Aviation Organization, and their transportation is subject to regulatory controls. For transport purposes, the term “infectious substance” is understood to include the term “etiologic agent.”

Transportation Regulations

International and domestic transport regulations for infectious substances are designed to prevent the release of these materials in transit to protect the public, workers, property, and the environment from the harmful effects that may occur from exposure to these materials. Protection is achieved through rigorous packaging requirements and hazard communication. Packages must be designed to withstand rough handling and other forces experienced in transportation, such as changes in air pressure and temperature, vibration, stacking, and moisture. Hazard communication includes shipping papers, labels, markings on the outside of packagings, and other information necessary to enable transport workers and emergency response personnel to correctly identify the material and respond efficiently in an emergency situation. In addition, shippers and carriers must be trained on these regulations so they can properly prepare shipments and recognize and respond to the risks posed by these materials.

Select agents include infectious substances that have been identified by the CDC and the USDA as having the potential to pose a severe threat to public health and safety. Persons who offer for transportation or transport select agents in commerce in the United States must develop and implement security plans for such transportation. A security plan must include an assessment of the possible transportation security risks for materials covered by the security plan and specific measures to reduce or eliminate the assessed risks. At a minimum, a security plan must include measures to address those risks associated with personnel security, en route security, and unauthorized access.

Regulations

Department of Transportation. 49 CFR Part 171-180, Hazardous Materials Regulations. Applies to the shipment of infectious substances in commercial transportation within the United States. Information on these regulations is available at: <http://www.phmsa.dot.gov/hazmat>.

United States Postal Service (USPS). 39 CFR Part 20, International Postal Service (International Mail Manual), and Part 111, General Information on Postal Service (Domestic Mail Manual). Regulations on transporting infectious substances through the USPS are codified in Section 601.10.17 of the Domestic Mail Manual and Section 135 of the International Mail Manual. A copy of the Domestic and International Mail Manuals may be obtained from the U.S. Government Printing Office by calling Monday through Friday, 7:30 a.m. – 9:00 p.m. EST: (202) 512-1800; toll free (866) 512-1800; or at the USPS Web site: <http://bookstore.gpo.gov/>.

Occupational Health and Safety Administration (OSHA). 29 CFR Part 1910.1030, Occupational Exposure to Bloodborne Pathogens. These regulations provide minimal packaging and labeling for blood and body fluids when transported within a laboratory or outside of it. Information may be obtained from your local OSHA office or at the OSHA Web site: <http://www.osha.gov>.

Technical Instructions for the Safe Transport of Dangerous Goods by Air (Technical Instructions). International Civil Aviation Organization (ICAO). Applies to the shipment of infectious substances by air and is recognized in the United States and by most countries worldwide. A copy of these regulations may be obtained from the ICAO Document Sales Unit at (514) 954-8022, fax: (514) 954-6769; e-mail: sales_unit@icao.int; or from the ICAO Web site: <http://www.icao.int>.

Dangerous Goods Regulations. International Air Transport Association (IATA). These regulations are issued by an airline association, are based on the ICAO Technical Instructions, and are followed by most airline carriers. A copy of these regulations is available at: <http://www.iata.org/index.htm> or <http://www.who.int/en/>; or by contacting the IATA Customer Care office at: telephone: +1 (514) 390 6726; fax: +1 (514) 874 9659; for Canada and USA (800) 716-6326 (toll free); Europe, Africa and Middle East +41 (22) 770 2751; fax: +41 (22) 770 2674; TTY: YMQTPXB, or e-mail: custserv@iata.org.

Transfers

Regulations governing the transfer of biological agents are designed to ensure that possession of these agents is in the best interest of the public and the nation. These regulations require documentation of personnel, facilities, justification of need and pre-approval of the transfer by a federal authority. The following regulations apply to this category:

Importation of Etiologic Agents of Human Disease. 42 CFR Part 71 Foreign Quarantine. Part 71.54 Etiological Agents, Hosts and Vectors. This regulation requires an import permit from the CDC for importation of etiologic agents, hosts or vectors of human disease. The regulation, application form, and additional guidance is available at the CDC Web site: <http://www.cdc.gov/od/eaipp>.

Completed application forms may be submitted to the CDC Etiologic Agent Import Permit Program by fax: (404) 718-2093, or by mail:

Centers for Disease Control and Prevention
Etiologic Agent Import Permit Program
1600 Clifton Road, N.E., Mailstop A-46
Atlanta, GA 30333

Importation of select agents or toxins into the U.S. also requires the intended recipient to be registered with the Select Agent Program and submit an APHIS/ CDC Form 2 to obtain approval to import the select agent or toxin prior to each importation event (see 42 CFR 73 and/or 9 CFR 121). More information regarding select agents and toxins is available at: www.selectagents.gov.

Importation of Etiologic Agents of Livestock, Poultry and Other Animal Diseases and Other Materials Derived from Livestock, Poultry or Other Animal. 9 CFR Parts 122. Organisms and Vectors. The USDA, APHIS, Veterinary Services (VS) requires that a permit be issued prior to the importation or domestic transfer (interstate movement) of etiologic disease agents of livestock, poultry, other animals. Information may be obtained at (301) 734-5960, or from the USDA Web site: http://www.aphis.usda.gov/animal_health. Completed permit applications may be submitted electronically at: http://www.aphis.usda.gov/permits/learn_epermits.shtml; or by fax to (301) 734-3652; or by mail to:

USDA APHIS VS
National Center for Import and Export
4700 River Road
Unit 2, Mailstop 22, Cubicle 1A07
Riverdale, MD 20737

Importation of select agents into the United States also requires the intended recipient to be registered with the Select Agent Program and submit an APHIS/ CDC Form 2 to obtain approval to import the select agent or toxin prior to each importation event (see 42 CFR 73 and/or 9 CFR 121). More information regarding select agents and toxins is available at: http://www.aphis.usda.gov/programs/ag_selectagent/index.shtml.

Importation of Plant Pests 7 CFR Part 330. Federal Plant Pest Regulations; General; Plant Pests; Soil; Stone and Quarry Products; Garbage. This regulation requires a permit for movement into or through the United States, or interstate any plant pest or a regulated product, article, or means of conveyance in accordance with this part. Information can be obtained by calling (877) 770-5990 or at the USDA Web site: <http://www.aphis.usda.gov/permits>.

Export of Etiologic Agents of Humans, Animals, Plants and Related Materials; Department of Commerce (DoC); 5 CFR Parts 730 to 799. This regulation requires that exporters of a wide variety of etiologic agents of human, plant and animal

diseases, including genetic material, and products which might be used for culture of large amounts of agents, will require an export license. Information may be obtained by calling the DoC Bureau of Export Administration at (202) 482-4811, or at the DoC Web site: <http://www.ntis.gov/products/export-regs.aspx>; or at <http://www.access.gpo.gov/bis/index.html>; and <http://www.bis.doc.gov>.

Transfer of CDC Select Agents and Toxins. 42 CFR Part 73 Possession, Use, and Transfer of Select Agents and Toxins. The CDC regulates the possession, use, and transfer of select agents and toxins that have the potential to pose a severe threat to public health and safety. The CDC Select Agent Program registers all laboratories and other entities in the United States that possess, use, or transfer a select agent or toxin. Entities transferring or receiving select agents and toxins must be registered with the Select Agent Program and submit an APHIS/CDC Form 2 (see 42 CFR 73 and/or 9 CFR 121) to obtain approval prior to transfer of a select agent or toxin. The regulations, Select Agent Program forms, and additional guidance is available at the CDC Web site: www.selectagents.gov.

Transfer of USDA Select Agents and Toxins. 9 CFR Part 121 Possession, Use, and Transfer of Select Agents and Toxins. The USDA, APHIS, VS regulates the possession, use, and transfer of select agents and toxins that have the potential to pose a severe threat to animal health or animal products. The VS Select Agent Program oversees these activities and registers all laboratories and other entities in the U.S. that possess, use, or transfer a VS select agent or toxin. Entities transferring or receiving select agents and toxins must be registered with either the CDC or APHIS Select Agent Program, and submit an APHIS/CDC Form 2 (see 42 CFR 73 and/or 9 CFR 121) to obtain approval prior to transfer of a select agent or toxin. The regulations, Select Agent Program forms, and additional guidance is available at the APHIS Web site: http://www.aphis.usda.gov/programs/ag_selectagent/index.shtml.

Transfer of USDA Plant Pests

The movement of Plant Pests is regulated under two distinct and separate regulations: (1) 7 CFR Part 331. Agricultural Bioterrorism Protection Act of 2002; Possession, Use, and Transfer of Biological Agents and Toxins; and (2) 7 CFR Part 330 Federal Plant Pest Regulations; General; Plant Pests; Soil; Stone and Quarry Products; Garbage. The regulation found at 7 CFR Part 331 requires an approved Transfer Form (APHIS/CDC Form 2) prior to importation, interstate, or intrastate movement of a Select Agent Plant Pest. In addition, under 7 CFR Part 330, the movement of a Plant Pest also requires a permit for movement into or through the United States, or interstate any plant pest or a regulated product, article, or means of conveyance in accordance with this part. Information can be obtained by calling (301) 734-5960 or at the USDA Web site: http://www.aphis.usda.gov/programs/ag_selectagent/index.shtml.

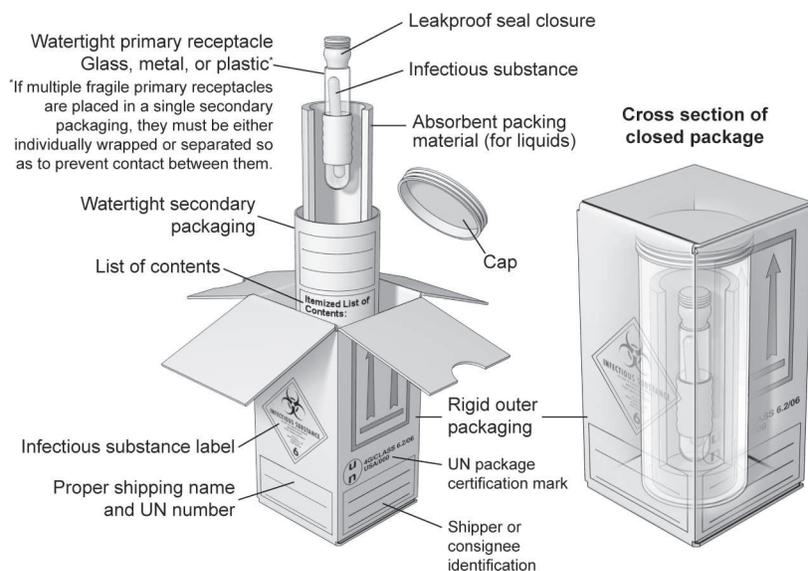
General DOT Packaging Requirements for Transport of Infectious Substances by Aircraft

The DOT packagings for transporting infectious substances by aircraft are required by domestic and international aircraft carriers, and are the basis for infectious substance packagings for motor vehicle, railcar, and vessel transport. The following is a summary of each packaging type and related transportation requirements.

Category A Infectious Substance (UN 2814 and UN 2900): Figure 1. A Category A material is an infectious substance that is transported in a form that is capable of causing permanent disability or life-threatening or fatal disease to otherwise healthy humans or animals when exposure to it occurs. An exposure occurs when an infectious substance is released outside of its protective packaging, resulting in physical contact with humans or animals. Category A infectious substances are assigned to identification number “UN 2814” for substances that cause disease in humans or in both humans and animals, or “UN 2900” for substances that cause disease in animals only.

Figure 1 shows an example of the UN standard triple packaging system for materials known or suspected of being a Category A infectious substance. The package consists of a watertight primary receptacle or receptacles; a watertight secondary packaging; for liquid materials, the secondary packaging must contain absorbent material in sufficient quantities to absorb the entire contents of all primary receptacles; and a rigid outer packaging of adequate strength for its capacity, mass, and intended use. Each surface of the external dimension of the packaging must be 100 mm (3.9 inches) or more. The completed package must pass specific performance tests, including a drop test and a water-spray test, and must be capable of withstanding, without leakage, an internal pressure producing a pressure differential of not less than 95 kPa (0.95 bar, 14 psi). The completed package must also be capable of withstanding, without leakage, temperatures in the range of -40°C to +55°C (-40°F to 131°F). The completed package must be marked “Infectious substances, affecting humans, UN 2814” or “Infectious substances, affecting animals, UN 2900” and labeled with a Division 6.2 (infectious substance) label. In addition, the package must be accompanied by appropriate shipping documentation, including a shipping paper and emergency response information.

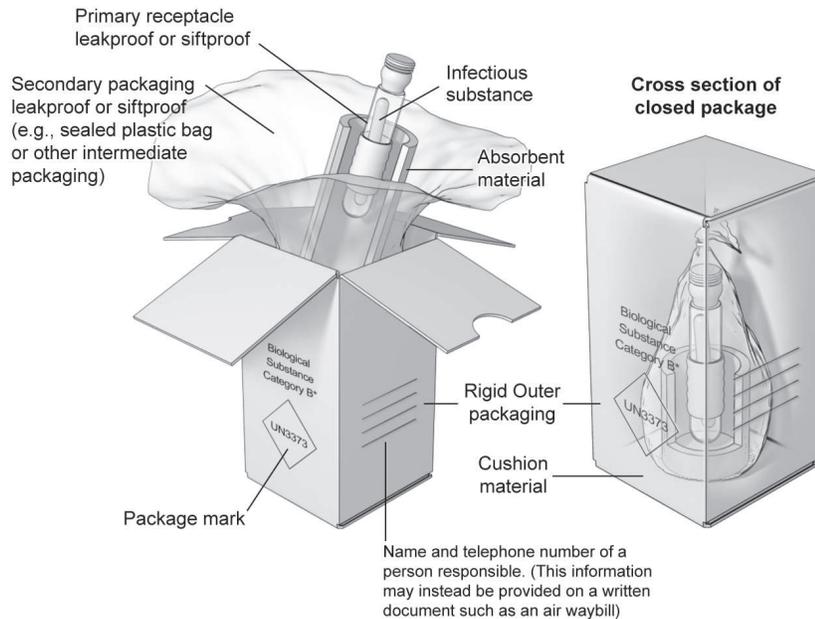
Figure 1. A Category A UN Standard Triple Packaging



Biological specimen, Category B (UN 3373): Figure 2. (previously known as Clinical specimen and Diagnostic Specimen). A Category B infectious substance is one that does not meet the criteria for inclusion in Category A. A Category B infectious substance does not cause permanent disability or life-threatening or fatal disease to humans or animals when exposure to it occurs. The proper shipping name for a Category B infectious substance, "Biological specimen, Category B," is assigned to identification number "UN 3373." The proper shipping names "Diagnostic specimen" and "Clinical specimen" may no longer be used (as of January 1, 2007).

Figure 2 shows an example of the triple packaging system for materials known or suspected of containing a Category B infectious substance. A Category B infectious substance must be placed in a packaging consisting of a leak proof primary receptacle, leak proof secondary packaging, and rigid outer packaging. At least one surface of the outer packaging must have a minimum dimension of 100 mm by 100 mm (3.9 inches). The packaging must be of good quality and strong enough to withstand the shocks and loadings normally encountered during transportation. For liquid materials, the secondary packaging must contain absorbent material in sufficient quantities to absorb the entire contents of all primary receptacles. The primary or secondary packaging must be capable of withstanding, without leakage, an internal pressure producing a pressure differential of 95 kPa. The package must be constructed and closed to prevent any loss of contents that might be caused under normal transportation conditions by vibration or changes in temperature, humidity, or pressure. The completed package must be capable of passing a 1.2-meter (3.9 feet) drop test. The package must be marked with a diamond-shaped marking containing the identification number "UN 3373" and with the proper shipping name "Biological substance, Category B." In addition, the name, address, and telephone number of a person knowledgeable about the material must be provided on a written document, such as an air waybill, or on the package itself.

Figure 2. A Category B Non-specification Triple Packaging



Appendix D—Agriculture Pathogen Biosafety

The contents of this Appendix were provided by USDA. All questions regarding its contents should be forwarded to the USDA.

Contents

- I. Introduction
- II. BSL-3-Ag
- III. BSL-3, Enhanced
- IV. Pathogens of Veterinary Significance
- V. Summaries of Selected Agriculture Agents
- VI. Additional information

I. Introduction

Risk assessment and management guidelines for agriculture differ from human public health standards. Risk management for agriculture research is based on the potential economic impact of animal and plant morbidity, and mortality, and the trade implications of disease. Agricultural guidelines take this difference into account. Worker protection is important but great emphasis is placed on reducing the risk of agent escape into the environment. This Appendix describes the facility parameters and work practices of what has come to be known as BSL-3-Ag. BSL-3-Ag is unique to agriculture because of the necessity to protect the environment from an economic, high risk pathogen in a situation where studies are conducted employing large agricultural animals or other similar situations in which the ***facility barriers now serve as primary containment***. Also described are some of the enhancements beyond BSL-3 that may be required by USDA/APHIS when working in the laboratory or vivarium with veterinary agents of concern. This Appendix provides guidance and is not regulatory nor is it meant to describe policy. Conditions for approval to work with specific agricultural agents are provided at the time USDA/APHIS permits a location to work with an agent.

II. BSL-3-Ag for Work with Loose-housed Animals

In agriculture, special biocontainment features are required for certain types of research involving high consequence livestock pathogens in animal species or other research where the room provides the primary containment. To support such research, USDA has developed a special facility designed, constructed and operated at a unique animal containment level called BSL-3-Ag. Using the containment features of the standard ABSL-3 facility as a starting point, BSL-3-Ag facilities are specifically designed to protect the environment by including almost all of the features ordinarily used for BSL-4 facilities as enhancements. All BSL-3-Ag containment spaces must be designed, constructed and certified as primary containment barriers.

The BSL-3-Ag facility can be a separate building, but more often, it is an isolated zone within a facility operating at a lower biosafety level, usually at BSL-3. This isolated zone has strictly controlled access with special physical security measures and functions on the “box within a box” principle. All BSL-3-Ag facilities that cannot readily house animals in primary containment devices require the features for an ABSL-3 facility with the following enhancements typical of BSL-4 facilities:

1. Personnel change and shower rooms that provide for the separation of laboratory clothing from animal facility clothing and that control access to the containment spaces. The facility is arranged so that personnel ingress and egress are only through a series of rooms consisting of: a ventilated vestibule with compressible gaskets on the two doors, a “clean” change room outside containment, a shower room at the non-containment/containment boundary, and a “dirty” change room within containment. Complete animal facility clothing (including undergarments, pants and shirts or jump suits, and shoes and gloves) is typically provided in the “dirty” change room, and put on by personnel before entering the research areas. In some facilities, complete animal facility clothing and personal protective equipment are provided in the “clean” change room, where they can be stored and stowed for use without entry into containment. When leaving a BSL-3-Ag animal space that acts as the primary barrier and that contains large volumes of aerosols containing highly infectious agents (an animal room, necropsy room, carcass disposal area, contaminated corridor, etc.), personnel usually would be required to remove “dirty” lab clothing, take a shower, and put on “clean” lab clothing immediately after leaving this high risk animal space and before going to any other part of the BSL-Ag facility. When leaving the facility, these personnel would take another shower at the access control shower and put on their street clothing. Soiled clothing worn in a BSL-3-Ag space is autoclaved before being laundered. Personnel moving from one space within containment to another will follow the practices and procedures described in the biosafety manual specifically developed for the particular facility and adopted by the laboratory director.
2. Access doors are self closing and lockable. Emergency exit doors are provided, but are locked on the outside against unauthorized use. The architect or engineer shall consider the practicality of providing vestibules at emergency exits.
3. Supplies, materials and equipment enter the BSL-3-Ag space only through an airlock, fumigation chamber, an interlocked and double-door autoclave or shower.

4. Double-door autoclaves engineered with bioseals are provided to decontaminate laboratory waste passing out of the containment area. The double doors of the autoclaves must be interlocked so that the outer door can be opened only after the completion of the sterilizing cycle, and to prevent the simultaneous opening of both doors. All double door autoclaves are situated through an exterior wall of the containment area, with the autoclave unit forming an airtight seal with the barrier wall and the bulk of the autoclave situated outside the containment space so that autoclave maintenance can be performed conveniently. A gas sterilizer, a pass-through liquid dunk tank, or a cold gas decontamination chamber must be provided for the safe removal of materials and equipment that are steam or heat sensitive. Disposable materials must be decontaminated through autoclaving or other validated decontamination method followed by incineration.
5. Dedicated, single pass, directional, and pressure gradient ventilation systems must be used. All BSL-3-Ag facilities have independent air supply and exhaust systems that are operated to provide directional airflow and a negative air pressure within the containment space. The directional airflow within the containment spaces moves from areas of least hazard potential toward areas of greatest hazard potential. A visible means of displaying pressure differentials is provided. The pressure differential display/gauge can be seen inside and outside of the containment space, and an alarm sounds when the preset pressure differential is not maintained. The air supply and exhaust systems must be interlocked to prevent reversal of the directional airflow and positive pressurization of containment spaces in the event of an exhaust system failure.
6. Supply and exhaust air to and from the containment space is HEPA filtered. Exhaust air is discharged in such a manner that it cannot be drawn into outside air intake systems. The HEPA filters are outside of containment but are located as near as possible to the containment space to minimize the length of potentially contaminated air ducts. The HEPA filter housings are fabricated to permit scan testing of the filters in place after installation, and to permit filter decontamination before removal. Backup HEPA filter units are strongly recommended to allow filter changes without disrupting research. The most severe requirements for these modern, high level biocontainment facilities include HEPA filters arranged both in series and in parallel on the exhaust side, and parallel HEPA filters on the supply side of the HVAC systems serving “high risk” areas where large amounts of aerosols containing BSL-3-Ag agents could be expected (e.g., animal rooms, contaminated corridors, necropsy areas, carcass disposal facilities). For these high-risk areas, redundant supply and exhaust fans are recommended. The supply and exhaust air systems should be equipped with pre-filters (80-90% efficient) to prolong

the life of the HEPA filters. Air handling systems must provide 100% outside conditioned air to the containment spaces.

7. Liquid effluents from BSL-3-Ag areas must be collected and decontaminated in a central liquid waste sterilization system before disposal into the sanitary sewers. Typically, a heat decontamination system is utilized in these facilities and equipment must be provided to process, heat and hold the contaminated liquid effluents to temperatures, pressures and times sufficient to inactivate all biohazardous materials that reasonably can be expected to be studied at the facility in the future. The system may need to operate at a wide range of temperatures and holding times to process effluents economically and efficiently. Double containment piping systems with leak alarms and annular space decontaminating capability should be considered for these wastes. Effluents from laboratory sinks, cabinets, floors and autoclave chambers are sterilized by heat treatment. Under certain conditions, liquid wastes from shower rooms and toilets may be decontaminated by chemical treatment systems. Facilities must be constructed with appropriate basements or piping tunnels to allow for inspection of plumbing systems.
8. Each BSL-3-Ag containment space shall have its interior surfaces (walls, floors, and ceilings) and penetrations sealed to create a functional area capable of being certified as airtight. It is recommended that a pressure decay test be used (new construction only). Information on how to conduct a pressure decay test may be found within Appendix 9B of the ARS Facilities Design Manual (Policy and Procedure 242.1M-ARS; <http://www.afm.ars.usda.gov>). This requirement includes all interior surfaces of all animal BSL-3-Ag spaces, not just the surfaces making up the external containment boundary. All walls are constructed slab to slab, and all penetrations, of whatever type, are sealed airtight to prevent escape of contained agents and to allow gaseous fumigation for biological decontamination. This requirement prevents cross contamination between individual BSL-3-Ag spaces and allows gaseous fumigation in one space without affecting other spaces. Exterior windows and vision panels, if required, are breakage-resistant and sealed. Greenhouses constructed to meet the BSL-3-Ag containment level will undergo the following tests, or the latest subsequent standards: (a) an air infiltration test conducted according to ASTM E 283-91; (b) a static pressure water resistance test conducted according to ASTM E 331-93; and (c) a dynamic pressure water resistance test conducted according to AAMA 501.1-94.
9. All ductwork serving BSL-3-Ag spaces shall be airtight (pressure tested-consult your facility engineer for testing and certification details).
10. The hinges and latch/knob areas of all passage doors shall be sealed to airtight requirements (pressure decay testing).

11. All airlock doors shall have air inflated or compressible gaskets. The compressed air lines to the air inflated gaskets shall be provided with HEPA filters and check valves.
12. Restraining devices shall be provided in large animal rooms.
13. Necropsy rooms shall be sized and equipped to accommodate large farm animals.
14. Pathological incinerators, or other approved means, must be provided for the safe disposal of the large carcasses of infected animals. Redundancy and the use of multiple technologies need to be considered and evaluated.
15. HEPA filters must be installed on all atmospheric vents serving plumbing traps, as near as possible to the point of use, or to the service cock, of central or local vacuum systems, and on the return lines of compressed air systems. All HEPA filters are installed to allow in-place decontamination and replacement. All traps are filled with liquid disinfectant.
16. If BSCs are installed, they should be located such that their operation is not adversely affected by air circulation and foot traffic. Class II BSCs use HEPA filters to treat their supply and exhaust air. Selection of the appropriate type of Class II BSCs will be dependent upon the proposed procedures and type of reagents utilized. BSC selection should be made with input from a knowledgeable safety professional well versed on the operational limitations of class II biohazard cabinetry. Supply air to a Class III cabinet is HEPA filtered, and the exhaust air must be double filtered (through a cabinet HEPA and then through a HEPA in a dedicated building exhaust system) before being discharged to the atmosphere.

III. BSL-3 and ABSL-3 Plus Potential Facility Enhancements for Agriculture Agent Permitting

The descriptions and requirements listed above for BSL-3-Ag studies are based on the use of high-risk organisms in animal systems or other types of agriculture research where the facility barriers, usually considered secondary barriers, now act as primary barriers. Certain agents that typically require a BSL-3-Ag facility for research that utilizes large agricultural animals may be studied in small animals in an enhanced BSL-3 laboratory or enhanced ABSL-3 when the research is done within primary containment devices. In these situations, the facility no longer serves as the primary barrier as with the large animal rooms. Therefore, when manipulating high consequence livestock pathogens in the laboratory or small animal facility, facility design and work procedures must meet the requirements of BSL-3 or ABSL-3 with additional enhancements unique to agriculture. Agriculture enhancements are agent, site and protocol dependent. The facility may have personnel

enter and exit through a clothing change and shower room, have a double-door autoclave and/or fumigation chamber, HEPA filter supply and exhaust air, and a validated or approved system in place to decontaminate research materials and waste. Surfaces must be smooth to support wipe-down decontamination and penetrations should be sealed and the room capable of sealing in case gaseous decontamination is required. Because all work with infectious material is conducted within primary containment, there is no requirement for pressure decay testing the room itself.

The need for any potential agriculture enhancements is dependant upon a risk assessment. Therefore, after an assessment and in consultation with USDA/ APHIS, the required agriculture enhancement(s) may include:

1. Personnel change and shower rooms that provide for the separation of street clothing from laboratory clothing and that control access to the containment spaces. The facility is arranged so that personnel ingress and egress are only through a series of rooms (usually one series for men and one for women) consisting of: a ventilated vestibule with a "clean" change room outside containment, a shower room at the non-containment/containment boundary, and a "dirty" change room within containment. Complete laboratory clothing (including undergarments, pants and shirts or jump suits, and shoes and gloves) is provided in the "dirty" change room, and put on by personnel before entering the research areas. In some facilities, complete laboratory clothing and personal protective equipment are provided in the "clean" change room, where they can be stored and stowed for use without entry into containment. When leaving a BSL-3 enhanced space, personnel usually would be required to remove their "dirty" laboratory clothing, take a shower, and put on "clean" laboratory clothing immediately after leaving the BSL-3 enhanced space and before going to any other part of the facility. Soiled clothing worn in a BSL-3 enhanced space should be autoclaved before being laundered outside of the containment space. Personnel moving from one space within containment to another will follow the practices and procedures described in the biosafety manual specifically developed for the particular facility and adopted by the laboratory director.
2. Access doors to these facilities are self closing and lockable. Emergency exit doors are provided but are locked on the outside against unauthorized use. The architect or engineer shall consider the practicality of providing vestibules at emergency exits.
3. Supplies, materials and equipment enter the BSL-3 enhanced space only through the double-door ventilated vestibule, fumigation chamber or an interlocked and double-door autoclave.

4. Double-door autoclaves engineered with bioseals are provided to decontaminate laboratory waste passing out of the containment area. The double doors of the autoclaves must be interlocked so that the outer door can be opened only after the completion of the sterilizing cycle, and to prevent the simultaneous opening of both doors. All double door autoclaves are situated through an exterior wall of the containment area, with the autoclave unit forming an airtight seal with the barrier wall and the bulk of the autoclave situated outside the containment space so that autoclave maintenance can be performed conveniently. A gas sterilizer, a pass-through liquid dunk tank, or a cold gas decontamination chamber must be provided for the safe removal of materials and equipment that are steam or heat sensitive. All other materials must be autoclaved or otherwise decontaminated by a method validated to inactivate the agent before being removed from the BSL-3 enhanced space. Wastes and other materials being removed from the BSL-3 enhanced space must be disposed of through incineration or other approved process.
5. Dedicated, single pass, directional, and pressure gradient ventilation systems must be used. All BSL-3 enhanced facilities have independent air supply and exhaust systems operated to provide directional airflow and a negative air pressure within the containment space. The directional airflow within the containment spaces moves from areas of least hazard potential toward areas of greatest hazard potential. A visible means of displaying pressure differentials is provided. The pressure differential display/gauge can be seen inside and outside of the containment space, and an alarm sounds when the preset pressure differential is not maintained. Supply and exhaust air to and from the containment space is HEPA filtered, with special electrical interlocks to prevent positive pressurization during electrical or mechanical breakdowns.
6. The exhaust air is discharged in such a manner that it cannot be drawn into outside air intake systems. HEPA filters located outside of the containment barrier are located as near as possible to the containment space to minimize the length of potentially contaminated air ducts. The HEPA filter housings are fabricated to permit scan testing of the filters in place after installation, and to permit filter decontamination before removal. Backup parallel HEPA filter units are strongly recommended to allow filter changes without disrupting research. Air handling systems must provide 100% outside conditioned air to the containment spaces.
7. Contaminated liquid wastes from BSL-3 enhanced areas must be collected and decontaminated by a method validated to inactivate the agent being used before disposal into the sanitary sewers. Treatment requirement will be determined by a site-specific, agent-specific risk assessment. Floor drains are discouraged in ABSL-3 and BSL-3

agriculture enhanced laboratories lacking a liquid waste central sterilization system. If floor drains are present, they should be capped and sealed. Facilities should be constructed with appropriate basements or piping tunnels to allow for inspection of plumbing systems, if a central liquid waste sterilization system is used.

8. Each BSL-3 enhanced containment space shall have its interior surfaces (walls, floors, and ceilings) and penetrations sealed to create a functional area capable of being decontaminated using a gaseous or vapor phase method. All walls are contiguous with the floor and ceiling, and all penetrations, of whatever type, are sealed. Construction materials should be appropriate for the intended end use. Exterior windows and vision panels, if required, are breakage-resistant and sealed.
9. All exhaust ductwork prior to the HEPA exhaust filter serving BSL-3 enhanced spaces shall be subjected to pressure decay testing before acceptance of the facility for use. Consult your facility engineer for testing and commissioning details.

IV. Pathogens of Veterinary Significance

Some pathogens of livestock, poultry and fish may require special laboratory design, operation, and containment features. This may be BSL-3, BSL-3 plus enhancements or BSL-4 and for animals ABSL-2, ABSL-3 or BSL-3-Ag. The importation, possession, or use of the following agents is prohibited or restricted by law or by USDA regulations or administrative policies.

This Appendix does not cover manipulation of diagnostic samples; however, if a foreign animal disease agent is suspected, samples should be immediately forwarded to a USDA diagnostic laboratory (The National Veterinary Services Laboratories, Ames, IA or the Foreign Animal Disease Diagnostic Laboratory, Plum Island, NY). A list of agents and their requirements follows.

African horse sickness virus ^{a, b}	Louping ill virus ^a
African swine fever virus ^{a, b, c}	Lumpy skin disease virus ^{a, b, c}
Akabane virus ^b	Malignant catarrhal fever virus (exotic strains or alcelaphine herpesvirus type 1) ^b
Avian influenza virus (highly pathogenic) ^{a, b, c}	Menangle virus ^b
<i>Bacillus anthracis</i> ^{a, b}	<i>Mycobacterium bovis</i>
<i>Besnoitia besnoiti</i>	<i>Mycoplasma agalactiae</i>
Bluetongue virus (exotic) ^{a, b}	<i>Mycoplasma mycoides subsp. mycoides</i> (small colony type) ^{a, b, c}

Borna disease virus	<i>Mycoplasma capricolum</i> ^{a, b, c}
Bovine infectious petechial fever agent	Nairobi sheep disease virus (Ganjam virus)
Bovine spongiform encephalopathy prion ^b	Newcastle disease virus (velogenic strains) ^{a, b, c}
<i>Brucella abortus</i> ^{a, b}	Nipah virus ^{a, b, d}
<i>Brucella melitensis</i> ^{a, b}	Peste des petits ruminants virus (plague of small ruminants) ^{a, b, c}
<i>Brucella suis</i> ^{a, b}	Rift Valley fever virus ^{a, b, c}
<i>Burkholderia mallei</i> / <i>Pseudomonas mallei</i> (Glanders) ^{a, b}	Rinderpest virus ^{a, b, c}
<i>Burkholderia pseudomallei</i> ^{a, b}	Sheep pox virus ^{a, b}
Camelpox virus ^b	Spring Viremia of Carp virus
Classical swine fever virus ^{a, b, c}	Swine vesicular disease virus ^b
<i>Coccidioides immitis</i> ^b	Teschen disease virus ^a
<i>Cochliomyia hominivorax</i> (Screwworm)	<i>Theileria annulata</i>
<i>Coxiella burnetii</i> (Q fever) ^b	<i>Theileria lawrencei</i>
Ephemeral fever virus	<i>Theileria bovis</i>
<i>Ehrlichia (Cowdria) ruminantium</i> (heartwater) ^b	<i>Theileria hirci</i>
Eastern equine encephalitis virus ^{a, b}	<i>Trypanosoma brucei</i>
Foot and mouth disease virus ^{a, b, c}	<i>Trypanosoma congolense</i>
<i>Francisella tularensis</i> ^b	<i>Trypanosoma equiperdum</i> (dourine)
Goat pox ^{a, b}	<i>Trypanosoma evansi</i>
Hemorrhagic disease of rabbits virus	<i>Trypanosoma vivax</i>
Hendra virus ^{a, b, d}	Venezuelan equine encephalomyelitis virus ^{a, b}
<i>Histoplasma (Zymonema) farciminosum</i>	Vesicular exanthema virus
Infectious salmon anemia virus	Vesicular stomatitis virus (exotic) ^{a, b}
Japanese encephalitis virus ^{a, b}	Wesselsbron disease virus

Notes:

- ^a Export license required by Department of Commerce (See: <http://www.bis.doc.gov/index.htm>).
- ^b Agents regulated as Select Agents under the Bioterrorism Act of 2002. Possession of these agents requires registration with either the CDC or APHIS and a permit issued for interstate movement or importation by APHIS-VS. Most require BSL-3/ABSL-3 or higher containment (enhancements as described in this Appendix or on a case-by-case basis as determined by APHIS-VS).
- ^c Requires BSL-3-Ag containment for all work with the agent in loose-housed animals.
- ^d Requires BSL-4 containment for all work with the agent.

A USDA/APHIS import or interstate movement permit is required to obtain any infectious agent of animals or plants that is regulated by USDA/APHIS. An import permit is also required to import any livestock or poultry product such as blood, serum, or other tissues.

V. Summaries of Selected Agriculture Agents

African Swine Fever Virus (ASFV)

ASF is a tick-borne and contagious, febrile, systemic viral disease of swine.^{1,2,3} The ASF virus (ASFV) is a large (about 200 nm) lipoprotein-enveloped, icosahedral, double-stranded DNA virus in the family *Asfarviridae*, genus *Asfivirus*. This virus is quite stable and will survive over a wide range of pH. The virus will survive for 15 weeks in putrefied blood, three hours at 50°C, 70 days in blood on wooden boards, 11 days in feces held at room temperature, 18 months in pig blood held at 4°C, 150 days in boned meat held at 39°F, and 140 days in salted dried hams. Initially, domestic and wild pigs (Africa: warthog, bush pig, and giant forest hog; Europe: feral pig) were thought to be the only hosts of ASFV. Subsequently, researchers showed that ASFV replicates in *Ornithodoros* ticks and that there is transstadial, transovarial, and sexual transmission. ASF in wild pigs in Africa is now believed to cycle between soft ticks living in warthog burrows and newborn warthogs. *Ornithodoros* ticks collected from Haiti, the Dominican Republic, and southern California have been shown to be capable vectors of ASFV, but in contrast to the African ticks, many of the ticks from California died after being infected with ASFV. Because ASFV-infected ticks can infect pigs, ASFV is the only DNA virus that can qualify as an arbovirus.

Even though the soft tick has been shown to be a vector (and in Africa probably the reservoir of ASFV), the primary method of spread from country to country has been through the feeding of uncooked garbage containing ASFV-infected pork scraps to pigs.

Aerosol transmission is not important in the spread of ASF. Because ASFV does not replicate in epithelial cells, the amount of virus shed by an ASF-infected pig is much less than the amount of virus shed by a hog-cholera-infected pig. The blood of a recently infected pig contains a very high ASFV titer.

Laboratory Safety and Containment Recommendations

Humans are not susceptible to ASFV infection. The greatest risk of working with the virus is the escape of the organism into a susceptible pig population, which would necessitate USDA emergency procedures to contain and eradicate the disease.

ASF is considered a foreign animal disease in the United States. Due to the highly contagious nature of the agent and the severe economic consequences of disease in the United States, this organism should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA and *in vivo* in a

USDA-approved BSL-3-Ag facility for loosely housed animals. Special consideration should be given to infected vector control.

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

African Horse Sickness Virus (AHSV)

AHSV is a member of genus *Orbivirus* in the family *Reoviridae*. Nine serotypes, numbers 1 – 9, are recognized. AHSV grows readily in embryonated chicken eggs, suckling mice, and a variety of standard cell cultures. AHSV infects and causes viremia in equids. Most horses die from the disease, about half of donkeys and most mules survive, but zebras show no disease. Viremias may last up to one month despite the rapid development of neutralizing antibodies. AHSV may cause disease in dogs, but these are not thought to be important in the natural history of the disease.^{4,5}

AHSV has been recognized in central Africa and periodically spreads to naive populations in South and North Africa, the Iberian Peninsula, the Middle East, Pakistan, Afghanistan, and India. AHSV is vectored by *Culicoides* species and perhaps by mosquitoes, biting flies, and ticks limiting viral spread to climates and seasons favorable to the vectors. At least one North American *Culicoides* species transmits AHSV. AHSV may infect carnivores that consume infected animals but these are not thought to be relevant to natural transmission to equids.

Occupational Infections

Encephalitis and uveochorioretinitis were observed in four laboratory workers accidentally exposed to freeze-dried modified live vaccine preparations. Although AHSV could not be conclusively linked to disease, all four had neutralizing antibodies. Encephalitis was documented in experimentally infected monkeys.

Laboratory Safety and Containment Recommendations

Virus may be present in virtually any sample taken from an infected animal, but the highest concentrations are found in spleen, lung, and lymph nodes. The only documented risk to laboratory workers involves aerosol exposure to large amounts of vaccine virus. AHSV is unusually stable in blood or serum stored at 4°C.

AHS is considered a foreign animal disease in the United States. Due to the severe economic consequences of disease presence in the United States, this organism should only be handled *in vitro* in a BSL-3 laboratory with

enhancements as required by the USDA and *in vivo* in a USDA-approved ABSL-3 animal facility with enhancements. Blood, serum, or tissues taken from equids in areas where AHSV exists are potential means of transmitting the agent long distances. Special consideration should be given to infected vector containment.

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Akabane Virus (AKAV)

AKAV is a member of the genus *Orthobunyavirus* in the Simbu serogroup of the family *Bunyaviridae*. The Simbu serogroup also includes Aino, Peaton, and Tinaroo viruses that can cause similar disease. Experimental infection of pregnant hamsters leads to death of the fetus. This virus grows and causes disease in chick embryos. Isolated in suckling mice and hamster lung cell cultures, AKAV is an important cause of disease in ruminants. The virus does not cause overt disease in adults but infects the placenta and fetal tissues in cattle, sheep, and goats to cause abortions, stillbirths, and congenital malformations. The broad range of clinical signs in the fetus is related primarily to central nervous system damage that occurs during the first trimester of pregnancy.^{6,7}

AKAV is not known to infect or cause disease in humans; concern focuses only on effects to agriculture and wildlife. Common names of disease include congenital arthrogryposis-hydranencephaly syndrome, Akabane disease, acorn calves, silly calves, curly lamb disease, curly calf disease, and dummy calf disease. The host range of naturally occurring Akabane disease appears limited to cattle, sheep, swine, and goats but other animals including swine and numerous wildlife species become infected. AKAV is an Old World virus, being found in Africa, Asia, and Australia. Disease is unusual in areas where the virus is common because animals generally become immune before pregnancy. AKAV spreads naturally only in gnat and mosquito insect vectors that become infected after feeding on viremic animals.

Laboratory Safety and Containment Recommendations

AKAV may be present in blood, sera, and tissues from infected animals, as well as vectors from endemic regions. Parenteral injection of these materials into naive animals and vector-borne spread to other animals represents a significant risk to agricultural interests.

Akabane disease is considered a foreign animal disease in the United States. Due to the severe economic consequences of disease presence in the United States, this organism should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA and *in vivo* in a USDA-approved ABSL-3 animal facility with enhancements. Special consideration should be given to infected vector containment.

Specials Issues

Although it is virtually certain AKAV will grow and cause disease in New World livestock, it is not known if it will cause viremias in New World wildlife high enough to infect vectors, if it can be vectored by New World insects, or if it will cause disease in New World wildlife. Because fetal disease may not become evident until months after virus transmission, an introduction into a new ecosystem may not be recognized before the virus has become firmly entrenched.

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Bluetongue Virus (BTV)

BTV is a member of the family *Reoviridae*, genus *Orbivirus*. There are 24 recognized serotypes numbered 1 through 24. BTV is notable for causing disease in sheep and cattle and is very similar to other orbiviruses that cause disease in deer (epizootic hemorrhagic disease of deer virus) and horses (AHSV), and a few that cause disease in man (Colorado tick fever virus and others). These viruses have dsRNA genomes distributed amongst 10 segments, enabling efficient reassortment. Growth on a wide variety of cultured cells is usually cytotoxic. Growth in animals results in viremia within three to four days that endures as long as 50 days despite the presence of high levels of neutralizing antibodies.^{8,9}

BTV infects all ruminants, but bluetongue disease is unusual except in sheep and is unpredictable even in sheep. Disease is evidenced by fever, hyperemia, swelling, and rarely erosions and ulceration of the buccal and nasal mucosa. Hyperemia of the coronary bands of the hooves may cause lameness. In the worst cases, the disease progresses through weakness, depression, rapid weight loss, prostration, and death. Maternal transmission to the fetus may cause abortion or fetal abnormalities in the first trimester. Bluetongue disease also occurs in cattle but is rarely diagnosed. BTV may infect fetal calves and result in abortion or fetal brain damage. The full host range of BTV is still unknown but includes wild ruminants, neonatal mice, dogs, and chicken embryos.

BTV infection occurs in tropical, subtropical, and temperate climates where the *Culicoides* vectors exist. Global warming may be expanding the geographic range of *Culicoides*, and therefore BTV, into higher latitudes. Most countries have a unique assortment of the 24 serotypes. For example, BTV serotypes 2, 10, 11, 13, and 17 are currently active in the United States, but serotypes 1, 3, 4, 6, 8, 12, and 17 were present in the Caribbean basin when last surveyed. Concern over the spread of individual serotypes by trade in animals and animal products has engendered costly worldwide trade barriers.

The primary natural mode of transmission is by *Culicoides* midges. Only a few of more than 1,000 species of *Culicoides* transmit BTV. A strong correlation between the vector species and the associated BTV suggests these viruses may have adapted to their local vector. Thus, BTV does not exist in areas such as the Northeast United States where the local *Culicoides* fails to transmit BTV. Virus is present in semen at peak of viremia, but this is not considered a major route of transmission. Because of the prolonged viremia, iatrogenic transmission is possible. Only modified-live (attenuated) virus vaccines are available and a vaccine for only one serotype is currently available in the United States.

Laboratory Safety and Containment Recommendations

BTV is not known to cause disease in humans under any conditions. BTV commonly enters the laboratory in blood samples. The virus is stable at -70°C and in blood or washed blood cells held at 4°C . Sera prepared from viremic animals may represent some risk if introduced parenterally into naive animals. Blood, sera, and bovine semen can carry BTV across disease control boundaries.

The most significant threat from BTV occurs when virus is inoculated parenterally into naive animals. If appropriate *Culicoides* are present, virus can be transmitted to other hosts. Therefore, BTV-infected animals must be controlled for the two-month period of viremia and protected against *Culicoides* by physical means and/ or performing experiments at least two months before local *Culicoides* emerge. Thus, BTV exotic to the United States should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA and *in vivo* in a USDA-approved ABSL-3 with enhancements. Special consideration should be given to infected vector containment. Special containment is only needed when working with serotypes of BTV that are exotic to the country or locality. BTV on laboratory surfaces is susceptible to 95% ethanol and 0.5% sodium hypochlorite solution.

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Classical Swine Fever Virus (Hog Cholera)

Classical swine fever is a highly contagious viral disease of swine that occurs worldwide in an acute, a subacute, a chronic, or a persistent form.¹⁰⁻¹² In the acute form, the disease is characterized by high fever, severe depression, multiple superficial and internal hemorrhages, and high morbidity and mortality. In the chronic form, the signs of depression, anorexia, and fever are less severe than in the acute form, and recovery is occasionally seen in mature animals. Transplacental infection with viral strains of low virulence often results in persistently infected piglets, which constitute a major cause of virus dissemination to noninfected farms. Although minor antigenic variants of classical swine fever virus (CSFV) have been reported, there is only one serotype. Hog cholera virus is a lipid-enveloped pathogen belonging to the family *Flaviviridae*, genus *Pestivirus*. The organism has a close antigenic relationship with the bovine viral diarrhea virus (BVDV) and the border disease virus (BDV). In a protein-rich environment, hog cholera virus is very stable and can survive for months in refrigerated meat and for years in frozen meat. The virus is sensitive to drying (desiccation) and is rapidly inactivated by a pH of less than 3 and greater than 11.

The pig is the only natural reservoir of CSFV. Blood, tissues, secretions and excretions from an infected animal contain virus. Transmission occurs mostly by the oral route, though infection can occur through the conjunctiva, mucous membrane, skin abrasion, insemination, and percutaneous blood transfer (e.g., common needle, contaminated instruments). Airborne transmission is not thought to be important in the epizootiology of classical swine fever. Introduction of infected pigs is the principal source of infection in classical swine fever-free herds. Farming activities such as auction sales, livestock shows, visits by feed dealers, and rendering trucks also are potential sources of contagion. Feeding of raw or insufficiently cooked garbage is a potent source of hog cholera virus. During the warm season, insect vectors common to the farm environment may spread hog cholera virus mechanically. There is no evidence, however, that hog cholera virus replicates in invertebrate vectors.

Laboratory Safety and Containment Recommendations

Humans being are not susceptible to infection by CSFV. The greatest risk of working with these viruses is the escape of the organism into susceptible domestic or feral pig populations, which would necessitate USDA emergency procedures to contain and eradicate the diseases.

The virus is considered cause of a foreign animal disease in the United States. Due to the highly contagious nature of the agent and the severe economic consequences of disease presence in the United States, this organism should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA and *in vivo* in a USDA-approved BSL-3-Ag facility for loosely housed

animals. Laboratory workers should have no contact with susceptible hosts for five days after working with the agent.

Specials Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Contagious Bovine Pleuropneumonia Agent (CBPP)

CBPP is a highly infectious acute, subacute, or chronic disease, primarily of cattle, affecting the lungs and occasionally the joints, caused by *Mycoplasma mycoides mycoides*.¹³⁻¹⁵ Contagious bovine pleuropneumonia is caused by *M. mycoides mycoides* small-colony type (SC type). *M. mycoides mycoides* large-colony type is pathogenic for sheep and goats but not for cattle. *M. mycoides mycoides* (SC type) survives well only *in vivo* and is quickly inactivated when exposed to normal external environmental conditions. The pathogen does not survive in meat or meat products and does not survive outside the animal in nature for more than a few days. Many of the routinely used disinfectants will effectively inactivate the organism.

CBPP is predominantly a disease of the genus *Bos*; both bovine and zebu cattle are naturally infected. There are many reported breed differences with respect to susceptibility. In general, European breeds tend to be more susceptible than indigenous African breeds. In zoos, the infection has been recorded in bison and yak. Although it has been reported that the domestic buffalo (*Bubalus bubalis*) is susceptible, the disease is difficult to produce experimentally in this species.

CBPP is endemic in most of Africa. It is a problem in parts of Asia, especially India and China. Periodically, CBPP occurs in Europe, and outbreaks within the last decade have occurred in Spain, Portugal, and Italy. The disease was eradicated from the United States in the nineteenth century, and it is not present currently in the Western hemisphere.

CBPP is spread by inhalation of droplets from an infected, coughing animal. Consequently, relatively close contact is required for transmission to occur. Outbreaks usually begin as the result of movement of an infected animal into a naive herd. There are limited anecdotal reports of fomite transmission, but fomites are not generally thought to be a problem.

Laboratory Safety and Containment Recommendations

Humans are not susceptible to infection by CBPP. The greatest risk of working with these mycoplasma is the escape of the organism into susceptible domestic bovine populations, which would necessitate USDA emergency procedures to contain and eradicate the diseases.

CBPP is considered a foreign animal disease in the United States. Due to the highly contagious nature of the agent and the severe economic consequences of disease presence in the United States, this organism should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA and *in vivo* in a USDA-approved BSL-3-Ag facility for loosely housed animals.

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Contagious Caprine Pleuropneumonia Agent (CCPP)

CCPP is an acute highly contagious disease of goats caused by a mycoplasma and characterized by fever, coughing, severe respiratory distress, and high mortality.¹⁶⁻¹⁸ The principal lesion at necropsy is fibrinous pleuropneumonia. The causative agent of CCPP is considered to be *M. mycoides capri* (type strain PG-3) or a new mycoplasma *M. capricolum* subsp. *capripneumoniae* (designated F-38).¹⁹⁻²¹ Neither of these agents occurs in North America.

M. mycoides mycoides has also been isolated from goats with pneumonia. This agent (the so-called large colony or LC variant of *M. mycoides mycoides*) usually produces septicemia, polyarthritis, mastitis, encephalitis, conjunctivitis, hepatitis, or pneumonia in goats. Some strains of this agent (LC variant) will cause pneumonia closely resembling CCPP, but the agent is not highly contagious and is not considered to cause CCPP. It does occur in North America. *M. capricolum capricolum*, a goat pathogen commonly associated with mastitis and polyarthritis in goats, can also produce pneumonia resembling CCPP, but it usually causes severe septicemia and polyarthritis. This agent (which does occur in the United States) is closely related to mycoplasma F-38 but can be differentiated from it using monoclonal antibodies.

CCPP is a disease of goats, and where the classical disease has been described, only goats were involved in spite of the presence of sheep and cattle. Mycoplasma F-38, the probable cause of the classic disease, does not cause disease in sheep or cattle. *M. mycoides capri*, the other agent considered a

cause of CCPP, will result in a fatal disease in experimentally inoculated sheep and can spread from goats to sheep. It is however, not recognized as a cause of natural disease in sheep.

CCPP has been described in many countries of Africa, the Middle East, Eastern Europe, the former Soviet Union, and the Far East. It is a major scourge in many of the most important goat-producing countries in the world and is considered by many to be the world's most devastating goat disease.

CCPP is transmitted by direct contact through inhalation of infective aerosols. Of the two known causative agents, F-38 is far more contagious. Outbreaks of the disease often occur after heavy rains (e.g., after the monsoons in India) and after cold spells. This is probably because recovered carrier animals start shedding the mycoplasmas after the stress of sudden climatic change. It is believed that a long-term carrier state may exist.

Laboratory Safety and Containment Recommendations

Humans are not susceptible to infection by the agent that causes CCPP. The greatest risk of working with this mycoplasma is the escape of the organism into susceptible domestic caprine populations, which would necessitate USDA emergency procedures to contain and eradicate the diseases.

CCPP is considered a foreign animal disease in the United States. Due to the highly contagious nature of the agent and the severe economic consequences of disease in the United States, this organism should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA and *in vivo* in a USDA-approved BSL-3-Ag facility for loosely housed animals.

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Foot and Mouth Disease Virus (FMD)

FMD is a severe, highly communicable viral disease of cloven-hoofed animals (cattle, swine, sheep, and goats), causing fever, malaise, vesicular lesions in affected livestock and in some cases death in young animals due to myocardial lesions.²² It can also affect a variety of wild ruminants (e.g., deer, bison). FMD is one of the most devastating diseases of livestock, causing large economic losses when introduced to FMD-free countries. The etiologic agent, FMD virus (FMDV), is a member of the *aphtovirus* genus, family *picornaviridae* with seven serotypes

(A, O, C, Asia1, SAT1, SAT2 and SAT3).²³ Humans are considered accidental hosts for FMDV and rarely become infected or develop clinical disease. Historically, humans have been exposed to large quantities of FMDV both during natural outbreaks among large herds of animals and in laboratory settings. Despite this, there has been an extremely low incidence of human infections reported and many have been anecdotal. Reports of fever, headaches and vesicles in the skin (especially at an accidental inoculation site) and oral mucosa have been associated with documented FMDV infections. The symptoms can be easily mistaken with those of Hand, Foot and Mouth Disease caused by coxsackie A viruses. On the other hand, humans have been shown to carry virus in their throats for up to three days after exposure to aerosols from infected animals, potentially making them carriers of FMDV. Humans and their clothing and footwear have been implicated as fomites for transmission of FMDV during outbreaks. Therefore, most FMDV laboratories impose a five day period of contact avoidance with susceptible species for personnel working with the viruses.

Laboratory Safety and Containment Recommendations

Laboratory practices for FMDV are principally designed to prevent transmission to susceptible livestock, but also to protect workers. The greatest risk of working with FMD is the escape of the organism into susceptible animal populations, which would necessitate USDA emergency procedures to contain and eradicate the disease.

The virus is considered a cause of a foreign animal disease in the United States. Due to the highly contagious nature and the severe economic consequences of disease presence in the United States, this virus should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA (see Section IV of this Appendix) and *in vivo* in USDA-approved BSL-3-Ag animal facilities. Infected animals are handled with standard protection (gloves, protective clothing). Change of clothing, personal showers and clearing of the throat and nose are required upon exiting contaminated areas in order to minimize virus transmission to susceptible species. Laboratory workers should have no contact with susceptible hosts for five days after working with the agent. In the United States, the Plum Island Animal Disease Center in New York is the only laboratory authorized to possess and work with this agent.

Special Issues

FMDV is a select agent. Possession, transfer and use of this agent requires application of procedures as detailed in the Agricultural Bioterrorism Protection Act of 2002 and codified in 9 CFR Part 121. All rules concerning the possession, storage, use, and transfer of select agents apply. Please review Appendix F of this document for further instructions regarding select agents. Law prohibits research with FMD on the United States mainland.

Heartwater Disease Agent (HD)

HD is a non-contagious disease of domestic and wild ruminants caused by *Ehrlichia ruminantium*.²⁴ *E. ruminantium* (formerly *Cowdria ruminantium*) is a member of the family *Rickettsiaceae* characterized by organisms that are obligate intracellular parasites. These organisms often persist in the face of an immune response due to their protected intracellular status. Rickettsias in natural conditions are found in mammals and blood-sucking arthropods. Ticks of the genus *Amblyomma* transmit *E. ruminantium*. HD occurs primarily in Africa, but has been recognized in the West Indies since the 1980's. The pathogen is transmitted by ticks of the genus *Amblyomma*, most importantly *A. variegatum* (tropical bont tick). This tick has wide distribution in Africa and is present on several Caribbean islands. Three North American tick species, *A. maculatum* (Gulf Coast tick), *A. cajennense*, and *A. dissimile*, can transmit the organism, causing concern that competent vectors could transmit *E. ruminantium* in the United States.

Severe HD comprises fever, depression, rapid breathing, and convulsions in cattle, sheep, goats and water buffalo. Whitetail deer also are susceptible to *E. ruminantium* infection and develop severe clinical disease. HD has not been diagnosed in the United States but occurs in numerous Caribbean islands, as well as in most countries of Africa, south of the Sahara Desert.

Laboratory Safety and Containment Recommendations

E. ruminantium can be found in whole blood, brain and experimentally in liver and kidney. It is not a human pathogen. Humans are not susceptible to infection with the agent that causes HD. The greatest risk of working with this agent is the escape of this organism (or infected ticks) into a susceptible domestic bovine population, which would necessitate USDA emergency procedures to contain and eradicate the disease.

HD is considered a foreign animal disease in the United States. *E. ruminantium* should be handled *in vitro* in BSL-3 laboratory facilities. Animal work should be conducted in ABSL-3 animal facilities or in ABSL-2 animal facilities with special modifications such as tick dams (where applicable).

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Infectious Salmon Anemia (ISA) Virus

ISA is a disease of Atlantic salmon (*Salmo salar*) caused by an orthomyxovirus in the family *Orthomyxoviridae*, genus *Isavirus*. Both wild and cultured Atlantic salmon are susceptible to infection, as are brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*) and herring. The first clinical cases of ISA in Atlantic salmon were reported from Norway in 1984. Since then, ISA has been observed in Canada (1996), Scotland (1998), Chile (1999), Faroe Islands (2000) and the U.S. (2001).^{25,26} There is significant molecular difference between virus isolates (i.e., “Norwegian”, “Scottish” and “North American”).²⁷ Clinical signs of ISA include severe anemia, swelling and hemorrhaging in the kidney and other organs, pale gills, protruding eyes, darkening of the posterior gut, fluid in the body cavity and lethargy. The infection is systemic and most noted in blood and mucus, muscle, internal organs and feces. The principal target organ for ISA virus (ISAV) is the liver. Signs usually appear two to four weeks after the initial infection.

Reservoirs of ISAV infection are unknown, but the spread of infection may occur due to the purchase of subclinically infected smolts, from farm to farm, and from fish slaughterhouses or industries where organic material (especially blood and processing water) from ISAV-infected fish is discharged without necessary treatment.²⁸

Laboratory Safety and Containment Recommendations

Humans are not susceptible to ISAV infection. The greatest risk of working with this virus is the escape of the organism into a susceptible fish population, which would necessitate USDA emergency procedures to contain and eradicate the disease.

ISA is considered a reportable disease in the United States. ISAV should be handled *in vitro* in BSL-2 laboratory facilities with enhancements as required by USDA. Animal inoculations should be handled in ABSL-3 animal facilities with special modifications as required. Recommended precautions include incineration of fish, tissues, blood and materials (gloves, laboratory coats, etc.) used in the collection and processing of fish samples. All surfaces exposed to potentially infected fish should be disinfected with 0.04 to 0.13% acetic acid, chlorine dioxide at 100 parts/million for five minutes or sodium hypochlorite 30 mg available chlorine/liter for two days or neutralized with sodium thiosulfate after three hours. General principles of laboratory safety should be practiced in handling and processing fish samples for diagnostic or investigative studies. Laboratory managers should evaluate the need to work with ISAV and the containment capability of the facility before undertaking work with the virus or suspected ISAV-infected fish.

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or

interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Lumpy Skin Disease (LSD)Virus

LSD is an acute to chronic viral disease of cattle characterized by skin nodules that may have inverted conical necrosis (sit fast) with lymphadenitis accompanied by a persistent fever.²⁹⁻³¹ The causative agent of LSD is a capripoxvirus in the family *Poxviridae*, genus *Capripoxvirus*. The prototype strain of LSD virus (LSDV) is the Neethling virus. LSDV is one of the largest viruses (170-260 nm by 300-450 nm) and there is only one serotype. The LSDV is very closely related serologically to the virus of sheep and goat pox (SGP) from which it cannot be distinguished by routine virus neutralization or other serological tests. The virus is very resistant to physical and chemical agents, persists in necrotic skin for at least 33 days and remains viable in lesions in air-dried hides for at least 18 days at ambient temperature.

LSD is a disorder of cattle. Other wild ungulates have not been infected during epizootics in Africa. Lumpy skin disease was first described in Northern Rhodesia in 1929. Since then, the disease has spread over most of Africa in a series of epizootics and most recently into the Middle East. Biting insects play the major (mechanical) role in the transmission of LSDV. Direct contact seems to play a minor role in the spread of LSD.

Laboratory Safety and Containment Recommendations

Human beings are not susceptible to infection by LSDV. The greatest risk of working with this virus is the escape of the organism into susceptible domestic animal populations, which would necessitate USDA emergency procedures to contain and eradicate the diseases.

Lumpy skin disease is considered a foreign animal disease in the United States. Due to the highly contagious nature of the agent and the severe economic consequences of disease in the United States, this organism should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA and *in vivo* in a USDA-approved BSL-3-Ag facility for loosely housed animals.

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Malignant Catarrhal Fever Virus (MCFV) (Exotic Strains)

Alcelaphine herpesvirus 1 (AIHV-1) is a herpesvirus of the *Rhadinovirus* genus in the *Gammaherpesvirinae* subfamily.³² Common names for AIHV-1 include wildebeest-associated malignant catarrhal fever virus (MCFV), African form MCFV, and exotic MCFV. It also was previously called bovine herpesvirus³. As a typical herpesvirus, AIHV-1 is a linear double-stranded DNA, enveloped virus. The virus can be propagated in certain primary or secondary cell cultures such as bovine thyroid and testis cells. The isolation of AIHV-1 requires the use of viable lymphoid cells from the diseased animal or cell-free virus in ocular/nasal secretions from wildebeest calves during a viral shedding period. Like other herpesviruses, AIHV-1 is fragile and quickly inactivated in harsh environments (for example, desiccation, high temperatures, and UV/sunlight), and by common disinfectants.

Wildebeest-associated MCF caused by AIHV-1 is also known as the African form of MCF, malignant catarrh, or snotsiekte (snotting sickness). The disease primarily affects many poorly adapted species of *Artiodactyla* that suffer very high case mortality (>95%) but low case morbidity (<7%). Wildebeest are the reservoir for AIHV-1 and the virus does not cause any significant disease in its natural host. Wildebeest-associated MCF primarily occurs in domestic cattle in Africa and in a variety of clinically susceptible ruminant species in zoological collections where wildebeest are present. Virtually all free-living wildebeest are infected with the virus and calves less than four months of age serve as the source of virus for transmission. The disease can be experimentally transmitted between cattle only by injection with infected viable cells from lymphoid tissues of affected animals. The disease cannot be transmitted by natural means from one clinically susceptible host to another, because there is essentially no cell-free virus in tissues or secretions of diseased animals. MCF is not a contagious disease.

Laboratory Safety and Containment Recommendations

There is no evidence that AIHV-1 can infect humans. Virus can be grown in several bovine cell lines at relatively low titers (ranging from 10³ to 10⁵ TCID₅₀). Infectivity in blood and tissues of affected animals is generally associated with viable lymphoid cells. The virus can be easily inactivated by wiping down surfaces with common disinfectants (such as bleach and sodium hypochlorite) and by autoclaving virus-contaminated materials.

This organism should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA and *in vivo* in a USDA-approved ABSL-3 animal facility with enhancements.

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or

interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Menangle Virus (MenV)

MenV caused a single outbreak of reproductive disease in an Australian swine operation. Clinical signs included stillborn, deformed, mummified piglets and a drop in the farrowing rate. Transmission between pigs has been postulated to be of a fecal-oral nature. A serological survey of fruit bats living near the swine operation revealed the presence of antibodies to MenV. Fruit bats are considered to be the natural host of the virus and their proximity to the affected premises led to an incidental infection in the pig population.^{33,34}

MenV is a member of the family *Paramyxoviridae*, subfamily *Paramyxovirinae*. Other members of this family include Hendra virus, Nipah virus and Tioman virus of which Hendra and Nipah have been found to be fruit bat-associated. This virus was isolated from stillborn piglets from a single outbreak of reproductive disease in a commercial swine operation in New South Wales, Australia in 1997.

Occupational Infections

There was serological evidence of MenV infection in two people that had close contact with infected pigs on the affected premises. They demonstrated clinical signs similar to those seen with influenza such as chills, fever, drenching sweats, headache and rash. Both workers recovered fully from their illness.

Laboratory Safety and Containment Recommendations

Laboratory practices for MenV are principally designed to prevent transmission to susceptible livestock, but also to protect workers. The greatest risk of working with MenV is the escape of the organism into susceptible animal populations, which would necessitate USDA emergency procedures to contain and eradicate the disease.

MenV is considered cause of a foreign animal disease in the United States and is a human pathogen. Due to the severe economic consequences of disease presence in the United States, this organism should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA and *in vivo* in a USDA-approved ABSL-3 animal facility with enhancements.

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Newcastle Disease (ND) Virus

ND is one of the most serious infectious diseases of poultry worldwide. It is primarily a respiratory disease, but nervous and enteric forms occur. All bird species are probably susceptible to infection with ND virus (NDV). The severity of the disease caused by any given NDV strain can vary from an unapparent infection to 100% mortality. The chicken is the most susceptible species. The biocontainment requirements for working with a particular strain are based on the virulence of the virus as determined by chicken inoculation and more recently by determination of amino acid sequence of the fusion protein cleavage site (as defined by the World Organization for Animal Health).³⁵ The virus is shed in respiratory secretions and in feces. Natural transmission among birds occurs by aerosol inhalation or by consumption of contaminated feed or water.^{36,37}

NDV is classified in the *Avulavirus* genus within the family *Paramyxoviridae*, subfamily *Paramyxovirinae*, in the order *Mononegavirales*. All NDV isolates are of a single serotype avian paramyxovirus type 1 (APMV-1) that includes the antigenic variants isolated from pigeons called pigeon paramyxovirus¹. All strains are readily propagated in embryonated chicken eggs and a variety of avian and mammalian cell cultures although special additives may be required to propagate the low virulence (lentogenic) viruses in some cell types.³⁵⁻³⁷

Occupational Infections

The most common infection is a self-limiting conjunctivitis with tearing and pain that develops within 24 hours of an eye exposure by aerosol, splash of infective fluids, or eye contact with contaminated hands. The occurrence of upper respiratory or generalized symptoms is rare.³⁸

Laboratory Safety and Containment Recommendations

NDV isolates may be recovered from any infected bird, but on occasion may be recovered from humans infected by contact with infected poultry. Humans treated with live NDV in experimental cancer therapies, or those who are exposed by laboratory contamination also are sources of the virus.³⁸ The greatest risk is for susceptible birds that may be exposed to NDV. If isolates of moderate to high virulence for chickens are used for human cancer therapies, those isolates are probably of greater risk for inadvertent exposure of birds and poultry than they are to the humans handling or being treated with those viruses.

ND (produced by moderate or highly virulent forms of the virus) is considered a foreign animal disease in the United States. Due to the highly contagious nature of the agent and the severe economic consequences of disease presence in the United States, this organism should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA and *in vivo* in a USDA-approved BSL-3-Ag facility for loosely housed animals. Laboratory workers should have no contact with susceptible hosts for five days

after working with the agent. Laboratory and animal studies with low virulence viruses or diagnostic accessions should be handled at BSL-2.

Special Issues

Velogenic strains of NDV are USDA select agents. Possession, transfer and use of this agent requires application of procedures as detailed in 9 CFR Part 121, Agricultural Bioterrorism Protection Act of 2002; Possession, Use and Transfer of Biological Agents and Toxins. All rules concerning the possession, storage, use, and transfer of select agents apply. Please review Appendix F of this document for further instructions regarding select agents. An importation or interstate movement permit for NDV must be obtained from USDA/APHIS/VS.

Peste Des Petits Ruminants Virus (PPRV)

PPRV causes disease variously termed stomatitis pneumoenteritis complex, kata, goat plague and pseudorinderpest. The virus affects sheep and especially goats, and is regarded as the most important disease of goats and possibly sheep in West Africa where they are a major source of animal protein. The disease is reported from sub-Saharan Africa north of the equator, the Arabian Peninsula, the Middle East, and the Indian Subcontinent. The virus has particular affinity for lymphoid tissues and epithelial tissue of the gastrointestinal and respiratory tracts, causing high fever, diphtheritic oral plaques, proliferative lip lesions, diarrhea, dehydration, pneumonia and death. In susceptible populations morbidity is commonly 90% and mortality 50-80%, but can reach 100%.³⁹

PPRV is a member of the family *Paramyxoviridae*, subfamily *Paramyxovirinae*, genus *Morbillivirus*, and species *peste-des-petits-ruminants virus*. Other important morbilliviruses include measles virus, rinderpest virus and canine distemper virus. As in all morbilliviruses, it is pleomorphic, enveloped, about 150 nm in diameter and contains a single molecule of linear, non-infectious, negative sense ssRNA.⁴⁰

The virus is environmentally fragile and requires close direct contact for transmission. Outbreaks typically occur after animal movement and commingling during seasonal migrations or religious festivals. Sources of virus include tears, nasal discharge, coughed secretions, and all secretions and excretions of incubating and sick animals. There is no carrier state, and animals recovering from natural infection have lifetime immunity.

Laboratory Safety and Containment Recommendations

PPRV is not known to infect humans in either laboratory or field settings. The greatest risk of working with PPRV is the escape of the organism into a

susceptible sheep or goat population, which would necessitate USDA emergency procedures to contain and eradicate the disease.

The virus is considered cause of a foreign animal disease in the United States. Due to the highly contagious nature of the agent and the severe economic consequences of disease presence in the United States, this organism should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA and *in vivo* in a USDA-approved BSL-3-Ag facility for loosely housed animals. Laboratory workers should have no contact with susceptible hosts for five days after working with the agent.

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Rinderpest Virus (RPV)

Rinderpest (RP) is a highly contagious viral disease of domestic cattle, buffaloes, sheep, goats and some breeds of pigs and a large variety of wildlife species.⁴¹ It is characterized by fever, oral erosions, diarrhea, lymphoid necrosis and high mortality. The disease is present in the Indian subcontinent, Near East and sub-Saharan Africa including Kenya and Somalia.

RPV is a single stranded RNA virus in the family *Paramyxoviridae*, genus *Morbillivirus*. It is immunologically related to canine distemper virus, human measles virus, peste des petits ruminants virus, and marine mammal morbilliviruses. There is only one serotype of RPV including several strains with a wide range of virulence.⁴²

RPV is a relatively fragile virus. The virus is sensitive to sunlight, heat, and most disinfectants. It rapidly inactivates at pH 2 and 12. Optimal pH for survival is 6.5 – 7.0. Glycerol and lipid solvents inactivate this virus.

Spread of RPV is by direct and indirect contact with infected animals. Aerosol transmission is not a significant means of transmission. Incubation period varies with strain of virus, dosage, and route of exposure. Following natural exposure, the incubation period ranges from 3 to 15 days but is usually 4 to 5 days.

Laboratory Safety and Containment Recommendations

There are no reports of RPV being a health hazard to humans. The greatest risk of working with RPV is the escape of the organism into susceptible animal populations, which would necessitate USDA emergency procedures to contain and eradicate the disease.

The virus is considered cause of a foreign animal disease in the United States. Due to the highly contagious nature of the agent and the severe economic consequences of disease presence in the United States, this organism should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA and *in vivo* in a USDA-approved BSL-3-Ag facility for loosely housed animals. Laboratory workers should have no contact with susceptible hosts for five days after working with the agent.

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Sheep and Goat Pox Virus (SGPV)

Sheep and goat pox (SGP) is an acute to chronic disease of sheep and goats characterized by generalized pox lesions throughout the skin and mucous membranes, a persistent fever, lymphadenitis, and often a focal viral pneumonia with lesions distributed uniformly throughout the lungs. Subclinical cases may occur. The virus that causes SGP is a capripoxvirus (SGPV), one of the largest viruses (170 – 260 nm by 300 – 450 nm) in the *Poxviridae* family, genus *Capripoxvirus*. It is closely related to the virus that causes lumpy skin disease. The SGPV is very resistant to physical and chemical agents.⁴³⁻⁴⁵

SGPV causes clinical disease in sheep and goats. The virus replicates in cattle but does not cause clinical disease. The disease has not been detected in wild ungulate populations. It is endemic in Africa, the Middle East, the Indian subcontinent, and much of Asia.

Contact is the main means of transmission of SGPV. Inhalation of aerosols from acutely affected animals, aerosols generated from dust contaminated from pox scabs in barns and night holding areas, and contact through skin abrasions either by fomites or by direct contact are the natural means of transmitting SGPV. Insect transmission (mechanical) is possible. The virus can cause infection experimentally by intravenous, intradermal, intranasal, or subcutaneous inoculation.

Laboratory Safety and Containment Recommendations

Humans are not susceptible to infection by these poxviruses. The greatest risk of working with these agents is the escape of the organism into susceptible domestic animal populations, which would necessitate USDA emergency procedures to contain and eradicate the diseases.⁴⁶

These viruses are considered cause of a foreign animal disease in the United States. Due to the highly contagious nature of the agent and the severe economic consequences of disease presence in the United States, this organism should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA and *in vivo* in a USDA-approved ABSL-3 animal facility with enhancements.

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Spring Viremia of Carp Virus (SVCV)

Spring Viremia of Carp virus (SVCV) is a rhabdovirus in the family *Rhabdoviridae*, genus *Vesiculovirus* that infects a broad range of fish species and causes high mortality in susceptible hosts in cold water. It is a World Organization for Animal Health Office International des Épizooties (OIE) reportable disease. Infections have occurred in common and koi carp (*Cyprinus carpio*), grass carp (*Crenopharyngodon idellus*), silver carp (*Hypophthalmichthys molitrix*), bighead (*Aristichthys nobilis*), cruian carp (*Carassius carassius*), goldfish (*C. auratus*), roach (*Rutilus rutilus*), ide (*Leuciscus idus*), tench (*Tinca tinca*) and sheatfish (*Silurus glanis*). Long indigenous to Europe, the Middle East and Asia, the disease was reported recently in South and North America. In the spring of 2002, SVCV was isolated from koi carp farmed in North Carolina. That year the virus was detected in fish in several lakes and rivers in Wisconsin, including the Mississippi River. SVCV causes impairment in salt-water balance in fish resulting in edema and hemorrhages.

Reservoirs of SVCV are infected fish and carriers from either cultured, feral or wild fish populations.⁴⁷ Virulent virus is shed via feces, urine, and gill, skin and mucus exudates. Liver, kidney, spleen, gill and brain are the primary organs containing the virus during infection.⁴⁸ It is surmised that horizontal transmission occurs when waterborne virus enters through the gills. Vertical transmission may be possible, especially via ovarian fluids. This virus may remain infective for long periods of time in water or mud. Once the virus is established in a pond or farm, it may be difficult to eradicate without destruction of all fish at the farm.^{25,28,49}

Laboratory Safety and Containment Recommendations

Human beings are not susceptible to SVCV infection. The greatest risk of working with SVCV is the escape of the organism into a susceptible fish

population, which would necessitate USDA emergency procedures to contain and eradicate the disease.

SVC is considered a reportable disease in the United States. SVCV should be handled *in vitro* in BSL-2 laboratory facilities with enhancements as required by USDA. Animal inoculations should be handled in ABSL-3 animal facilities with special modifications as required. The OIE Diagnostic Manual for Aquatic Animal Disease has specifications for surveillance programs to achieve and maintain health status of aquaculture facilities.⁴⁸ Recommendations for preventing the disease and spread of disease include the use of a water source free of virus, disinfection of eggs and equipment, and proper disposal of dead fish.

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Swine Vesicular Disease Virus (SVDV)

Swine vesicular disease virus (SVDV) is classified in the genus *Enterovirus*, the family *Picornaviridae*, and is closely related to the human enterovirus coxsackievirus B5.⁵⁰ The virus is the causative agent of SVD, a contagious disease of pigs characterized by fever and vesicles with subsequent erosion in the mouth and on the snout, feet, and teats.^{51,52} The major importance of SVD is that it clinically resembles FMD, and any outbreaks of vesicular disease in pigs must be assumed to be FMD until proven otherwise by laboratory tests.

Occupational Infections

SVDV can cause an “influenza-like” illness in man¹ and human infection has been reported in laboratory personnel working with the virus.^{53,54} The virus may be present in blood, vesicular fluid, and tissues of infected pigs. Direct and indirect contacts of infected materials, contaminated laboratory surfaces, and accidental autoinoculation, are the primary hazards to laboratory personnel.

Laboratory Safety and Containment Recommendations

Laboratory practices for SVDV are principally designed to prevent transmission to susceptible livestock, but also to protect workers. Gloves are recommended for the necropsy and handling of infected animals and cell cultures. The greatest risk of working with SVD is the escape of the organism into susceptible animal populations, which would necessitate USDA emergency procedures to contain and eradicate the disease.⁵⁵

SVD is considered a foreign animal disease in the United States. Due to the severe economic consequences of disease presence in the United States, SVDV should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA and *in vivo* in a USDA-approved ABSL-3 animal facility with enhancements.

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

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VI. Additional Information:

U.S. Department of Agriculture
 Animal and Plant Health Inspection Service
 Veterinary Services, National Center for Import and Export
 4700 River Road, Unit 133
 Riverdale, Maryland 20737-1231
 Telephone: (301) 734-5960
 Fax: (301) 734-3256
 Internet: http://www.aphis.usda.gov/animal_health/permits

Further information on Plant Select Agents, or permits for field release of genetically engineered organisms may be obtained from:

U.S. Department of Agriculture Animal
 and Plant Health Inspection Service
 Plant Protection and Quarantine, Permits, Agricultural Bioterrorism
 4700 River Road, Unit 2
 Riverdale, Maryland 20737-1231
 Telephone: (301) 734-5960
 Internet: http://www.aphis.usda.gov/programs/ag_selectagent/index.shtml

Appendix E—Arthropod Containment Guidelines (ACG)

An ad hoc committee of concerned vector biologists including members of the American Committee Medical Entomology (ACME), a subcommittee of the American Society of Tropical Medicine and Hygiene (ASTMH), and other interested persons drafted the “Arthropod Containment Guidelines.” The ACG provide principles of risk assessment for arthropods of public health importance. The risk assessment and practices are designed to be consistent with the *NIH Guidelines* for recombinant DNA research and BMBL. Arthropods included are those that transmit pathogens; however, those arthropods that cause myiasis, infestation, biting, and stinging are not included. The ACG also specifically exclude most uses of *Drosophila* spp.

The ACG were published in *Vector Borne and Zoonotic Diseases*.¹ They are freely downloadable from www.liebertonline.com and at the AMCE Web site: www.astmh.org.

The ACG recommend biosafety measures specific for arthropods of public health importance considering that:

- Arthropods present unique containment challenges not encountered with microbial pathogens.
- Arthropod containment has not been covered specifically in BMBL or the NIH Guidelines.

The ACG contain two sections of greatest interest to most researchers:

1. The Principles of Risk Assessment that discusses arthropods in the usual context (e.g., those known to contain a pathogenic agent, those with uncertain pathogens, and those with no agent).
2. They also consider the following:
 - Biological containment is a significant factor that reduces the hazards associated with accidental escape of arthropods.
 - Epidemiological context alters the risks of an escape and its impact on the location or site in which the work is performed.
 - The phenotype of the vector, such as insecticide resistance; and
 - genetically modified arthropods with an emphasis on phenotypic change.

Four Arthropod Containment Levels (ACL 1 – 4) add increasingly stringent measures and are similar to biosafety levels. The most flexible level is ACL-2 that covers most exotic and transgenic arthropods and those infected with pathogens requiring BSL-2 containment. Like BMBL, each level has the following form:

- standard practices;
- special practices;
- equipment (primary barriers);
- facilities (secondary barriers).

The ACG does not reflect a formal endorsement by ACME or ASTMH. The guidelines are subject to change based on further consideration of the requirements for containment of arthropods and vectors.

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Appendix F—Select Agents and Toxins

The *Public Health Security and Bioterrorism Preparedness and Response Act of 2002, Subtitle A of Public Law 107-188 (42 U.S.C. 262a)*, requires DHHS to regulate the possession, use, and transfer of biological agents or toxins (i.e., select agents and toxins) that could pose a severe threat to public health and safety. The *Agricultural Bioterrorism Protection Act of 2002, Subtitle B of Public Law 107-188 (7 U.S.C. 8401)*, requires the USDA to regulate the possession, use, and transfer of biological agents or toxins (i.e., select agents and toxins) that could pose a severe threat to animal or plant health, or animal or plant products. These Acts require the establishment of a national database of registered entities, and set criminal penalties for failing to comply with the requirements of the Acts. In accordance with these Acts, DHHS and USDA promulgated regulations requiring entities to register with the CDC or the APHIS if they possess, use, or transfer a select agent or toxin (42 CFR Part 73, 7 CFR Part 331, and 9 CFR Part 121). CDC and APHIS coordinate regulatory activities for those agents that would be regulated by both agencies (“overlap” select agents).

The Attorney General has the authority and responsibility to conduct electronic database checks (i.e., the security risk assessments) on entities that apply to possess, use, or transfer select agents, as well as personnel that require access to select agents and toxins. The FBI, Criminal Justice Information Services Division (CJIS), has been delegated authority for conducting these security risk assessments.

The regulations provide that, unless exempted, entities must register with CDC or APHIS if they possess, use, or transfer select agents or toxins. The current list of select agents and toxins is available on the CDC and APHIS Web sites (see below). The regulations set out a procedure for excluding an attenuated strain of a select agent or toxin and exemptions for certain products and for select agents or toxins identified in specimens presented for diagnosis, verification, or proficiency testing.

The regulations also contain requirements to ensure that the select agents and toxins are handled safely and secured against unauthorized access, theft, loss, or release. For example, entities and their personnel must undergo a security risk assessment by CJIS as part of their registration; entities must limit access to select agents and toxins and develop and implement biosafety, security, and incident response plans. In addition, all select agents or toxins must be transferred in accordance with the regulations and any theft, loss, or release of a select agent or toxin must be reported to CDC or APHIS.

For additional information concerning the select agent regulations, contact CDC or APHIS. Information is also available at the following Web sites: www.selectagents.gov; http://www.aphis.usda.gov/programs/ag_selectagent/index.shtml.

Appendix G—Integrated Pest Management (IPM)

IPM is an important part of managing a research facility. Many pests, such as flies and cockroaches, can mechanically transmit disease pathogens and compromise the research environment. Even the presence of innocuous insects can contribute to the perception of unsanitary conditions.

The most common approach to pest control has been the application of pesticides, either as a preventive or remedial measure. Pesticides can be effective and may be necessary as a corrective measure, but they have limited long-term effect when used alone. Pesticides also can contaminate the research environment through pesticide drift and volatilization.

To control pests and minimize the use of pesticides, it is necessary to employ a comprehensive program approach that integrates housekeeping, maintenance, and pest control services. This method of pest control is often referred to as IPM. The primary goal of an IPM program is to prevent pest problems by managing the facility environment to make it less conducive to pest infestation. Along with limited applications of pesticides, pest control is achieved through proactive operational and administrative intervention strategies to correct conditions that foster pest problems.

Prior to developing any type of IPM program, it is important to define an operational framework for IPM services that helps promote collaboration between IPM specialists and facility personnel. This framework should incorporate facility restrictions and operational and procedural issues into the IPM program. An effective IPM program is an integral part of the facility's management. An IPM policy statement should be included in the facility's standard operating procedures to increase awareness of the program.

Training sources for the principles and practices of structural (indoor) IPM programs are available through university entomology departments, county extension offices, the Entomological Society of America, state departments of agriculture, state pest control associations, the National Pest Control Association, suppliers of pest control equipment, and IPM consultants and firms. Several universities offer correspondence courses, short courses, and training conferences on structural pest management.

IPM is a strategy-based service that considers not only the cost of the services, but also the effectiveness of the program's components. Each IPM program is site-specific, tailored to the environment where applied.

Laboratory IPM services will be different from those in an office building or an animal care facility. Interrelated components of "Environmental pest management" follow.

Facility Design

IPM issues and requirements should be addressed in a research facility's planning, design, and construction. This provides an opportunity to incorporate features that help exclude pests, minimize pest habitat, and promote proper sanitation in order to reduce future corrections that can disrupt research operations.

Monitoring

Monitoring is the central activity of an IPM program and is used to minimize pesticide use. Traps, visual inspections, and staff interviews identify areas and conditions that may foster pest activity.

Sanitation and Facility Maintenance

Many pest problems can be prevented or corrected by ensuring proper sanitation, reducing clutter and pest habitat, and by performing repairs that exclude pests. Records of structural deficiencies and housekeeping conditions should be maintained to track problems and determine if corrective actions were completed and in a timely manner.

Communication

A staff member should be designated to meet with IPM personnel to assist in resolving facility issues that impact on pest management. Reports communicated verbally and in writing concerning pest activity and improvement recommendations for personnel, practices and facility conditions should be provided to the designated personnel. Facility personnel should receive training on pest identification, biology, and sanitation, which can promote understanding and cooperation with the goals of the IPM program.

Recordkeeping

A logbook should be used to record pest activity and conditions pertinent to the IPM program. It may contain protocols and procedures for IPM services in that facility, Material Safety Data Sheets on pesticides, pesticide labels, treatment records, floor plans, survey reports, etc.

Non-pesticide Pest Control

Pest control methods such as trapping, exclusion, caulking, washing, and freezing can be applied safely and effectively when used in conjunction with proper sanitation and structural repair.

Pest Control with Pesticides

Preventive applications of pesticides should be discouraged, and treatments should be restricted to areas of known pest activity. When pesticides are applied, the least toxic product(s) available should be used and applied in the most effective and safe manner.

Program Evaluation and Quality Assurance

Quality assurance and program review should be performed to provide an objective, ongoing evaluation of IPM activities and effectiveness to ensure that the program does, in fact, control pests and meet the specific needs of the facility program(s) and its occupants. Based upon this review, current IPM protocols can be modified and new procedures implemented.

Technical Expertise

A qualified entomologist can provide helpful technical guidance to develop and implement an IPM program. Pest management personnel should be licensed and certified by the appropriate regulatory agency.

Safety

IPM minimizes the potential of pesticide exposure to the research environment and the staff by limiting the scope of pesticide treatments.

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Appendix H—Working with Human, NHP and Other Mammalian Cells and Tissues

Although risk of laboratory infection from working with cell cultures in general is low, risk increases when working with human and other primate cells, and primary cells from other mammalian species. There are reports of infection of laboratory workers handling primary rhesus monkey kidney cells,¹ and the bloodborne pathogen risks from working with primary human cells, tissues and body fluids are widely recognized.^{2,3} OSHA has developed a bloodborne pathogens standard that should be applied to all work in the laboratory with human blood, tissues, body fluids and primary cell lines.⁴ Procedures have also been published to reduce contamination of cell cultures with microorganisms.^{5,6}

Potential Laboratory Hazards

Potential laboratory hazards associated with human cells and tissues include the bloodborne pathogens HBV, HIV, HCV, HTLV, EBV, HPV and CMV as well as agents such as *Mycobacterium tuberculosis* that may be present in human lung tissue. Other primate cells and tissues also present risks to laboratory workers.⁷ Cells immortalized with viral agents such as SV-40, EBV adenovirus or HPV, as well as cells carrying viral genomic material also present potential hazards to laboratory workers. Tumorigenic human cells also are potential hazards as a result of self-inoculation.⁸ There has been one reported case of development of a tumor from an accidental needle-stick.⁹ Laboratory workers should never handle autologous cells or tissues.¹ NHP cells, blood, lymphoid and neural tissues should always be considered potentially hazardous.

Recommended Practices

Each institution should conduct a risk assessment based on the origin of the cells or tissues (species and tissue type), as well as the source (recently isolated or well-characterized). Human and other primate cells should be handled using BSL-2 practices and containment. All work should be performed in a BSC, and all material decontaminated by autoclaving or disinfection before discarding.^{6,10,11,12} BSL-2 recommendations for personnel protective equipment such as laboratory coats, gloves and eye protection should be rigorously followed. All laboratory staff working with human cells and tissues should be enrolled in an occupational medicine program specific for bloodborne pathogens and should work under the policies and guidelines established by the institution's Exposure Control Plan.⁴ Laboratory staff working with human cells and tissues should provide a baseline serum sample, be offered hepatitis B immunization, and be evaluated by a health care professional following an exposure incident. Similar programs should be considered for work with NHP blood, body fluids, and other tissues.

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Appendix I—Guidelines for Work with Toxins of Biological Origin

Biological toxins comprise a broad range of poisons, predominantly of natural origin but increasingly accessible by modern synthetic methods, which may cause death or severe incapacitation at relatively low exposure levels.^{1,2} Laboratory safety principles are summarized herein for several toxins currently regulated as “Select Agent Toxins,” including BoNT, SE, ricin and selected LMW toxins. Additional details are provided in the agent summary statements.

General Considerations for Toxin Use

Laboratory work with most toxins, in amounts routinely employed in the biomedical sciences, can be performed safely with minimal risk to the worker and negligible risk to the surrounding community. Toxins do not replicate, are not infectious, and are difficult to transmit mechanically or manually from person to person. Many commonly employed toxins have very low volatility and, especially in the case of protein toxins, are relatively unstable in the environment; these characteristics further limit the spread of toxins.

Toxins can be handled using established general guidelines for toxic or highly-toxic chemicals with the incorporation of additional safety and security measures based upon a risk assessment for each specific laboratory operation.^{3,4} The main laboratory risks are accidental exposure by direct contamination of mouth, eyes or other mucous membranes; by inadvertent aerosol generation; and by needle-sticks or other accidents that may compromise the normal barrier of the skin.

Training and Laboratory Planning

Each laboratory worker must be trained in the theory and practice of the toxins to be used, with special emphasis on the nature of the practical hazards associated with laboratory operations. This includes how to handle transfers of liquids containing toxin, where to place waste solutions and contaminated materials or equipment, and how to decontaminate work areas after routine operations, as well as after accidental spills. The worker must be reliable and sufficiently adept at all required manipulations before being provided with toxin.

A risk assessment should be conducted to develop safe operating procedures before undertaking laboratory operations with toxins; suggested “pre-operational checklists” for working with toxins are available.⁴ For complex operations, it is recommended that new workers undergo supervised practice runs in which the exact laboratory procedures to be undertaken are rehearsed without active toxin. If toxins and infectious agents are used together, then both must be considered when containment equipment is selected and safety procedures are developed. Likewise, animal safety practices must be considered for toxin work involving animals.

Each laboratory that uses toxins should develop a specific chemical hygiene plan. The National Research Council has provided a review of prudent laboratory practices when handling toxic and highly toxic chemicals, including the development of chemical hygiene plans and guidelines for compliance with regulations governing occupational safety and health, hazard communication, and environmental protection.⁵

An inventory control system should be in place to account for toxin use and disposition. If toxins are stored in the laboratory, containers should be sealed, labeled, and secured to ensure restricted access; refrigerators and other storage containers should be clearly labeled and provide contact information for trained, responsible laboratory staff.

Laboratory work with toxins should be done only in designated rooms with controlled access and at pre-determined bench areas. When toxins are in use, the room should be clearly posted: "Toxins in Use—Authorized Personnel Only." Unrelated and nonessential work should be restricted from areas where stock solutions of toxin or organisms producing toxin are used. Visitors or other untrained personnel granted laboratory access must be monitored and protected from inadvertently handling laboratory equipment used to manipulate the toxin or organism.

Safety Equipment and Containment

Routine operations with dilute toxin solutions are conducted under BSL-2 conditions with the aid of personal protective equipment and a well-maintained BSC or comparable engineering controls.⁶ Engineering controls should be selected according to the risk assessment for each specific toxin operation. A certified BSC or chemical fume hood will suffice for routine operations with most protein toxins. Low molecular weight toxin solutions, or work involving volatile chemicals or radionucleotides combined with toxin solutions, may require the use of a charcoal-based hood filter in addition to HEPA filtration.

All work with toxins should be conducted within the operationally effective zone of the hood or BSC, and each user should verify the inward airflow before initiating work. When using an open-fronted fume hood or BSC, workers should wear suitable laboratory PPE to protect the hands and arms, such as laboratory coats, smocks, or coveralls and disposable gloves. When working with toxins that pose direct percutaneous hazards, special care must be taken to select gloves that are impervious to the toxin and the diluents or solvents employed. When conducting liquid transfers and other operations that pose a potential splash or droplet hazard in an open-fronted hood or BSC, workers should wear safety glasses and disposable facemask, or a face shield.

Toxin should be removed from the hood or BSC only after the exterior of the closed primary container has been decontaminated and placed in a clean secondary container. Toxin solutions, especially concentrated stock solutions, should be transported in leak/spill-proof secondary containers. The interior of the hood or BSC should be decontaminated periodically, for example, at the end of a series of related experiments. Until thoroughly decontaminated, the hood or BSC should be posted to indicate that toxins remain in use, and access should remain restricted.

Selected operations with toxins may require modified BSL-3 practices and procedures. The determination to use BSL-3 is made in consultation with available safety staff and is based upon a risk assessment that considers the variables of each specific laboratory operation, especially the toxin under study, the physical state of the toxin (solution or dry form), the total amount of toxin used relative to the estimated human lethal dose, the volume of the material manipulated, the methodology, and any human or equipment performance limitations.

Inadvertent Toxin Aerosols

Emphasis must be placed on evaluating and modifying experimental procedures to eliminate the possibility of inadvertent generation of toxin aerosols. Pressurized tubes or other containers holding toxins should be opened in a BSC, chemical fume hood, or other ventilated enclosure. Operations that expose toxin solutions to vacuum or pressure, for example sterilization of toxin solutions by membrane filtration, should always be handled in this manner, and the operator should also use appropriate respiratory protection. If vacuum lines are used with toxin, they should be protected with a HEPA filter to prevent entry of toxins into the line.

Centrifugation of cultures or materials potentially containing toxins should only be performed using sealed, thick-walled tubes in safety centrifuge cups or sealed rotors. The outside surfaces of containers and rotors should be routinely cleaned before each use to prevent contamination that may generate an aerosol. After centrifugation, the entire rotor assembly is taken from the centrifuge to a BSC to open it and remove its tubes.

Mechanical Injuries

Accidental needle-sticks or mechanical injury from “sharps” such as glass or metal implements pose a well-known risk to laboratory workers, and the consequences may be catastrophic for operations involving toxins in amounts that exceed a human lethal dose.

Only workers trained and experienced in handling animals should be permitted to conduct operations involving injection of toxin solutions using hollow-bore needles. Discarded needles/syringes and other sharps should be placed directly into properly labeled, puncture-resistant sharps containers, and decontaminated as soon as is practical.

Glassware should be replaced with plastic for handling toxin solutions wherever practical to minimize the risk of cuts or abrasions from contaminated surfaces. Thin-walled glass equipment should be completely avoided. Glass Pasteur pipettes are particularly dangerous for transferring toxin solutions and should be replaced with disposable plastic pipettes. Glass chromatography columns under pressure must be enclosed within a plastic water jacket or other secondary container.

Additional Precautions

Experiments should be planned to eliminate or minimize work with dry toxin (e.g., freeze-dried preparations). Unavoidable operations with dry toxin should only be undertaken with appropriate respiratory protection and engineering controls. Dry toxin can be manipulated using a Class III BSC, or with the use of secondary containment such as a disposable glove bag or glove box within a hood or Class II BSC. “Static-free” disposable gloves should be worn when working with dry forms of toxins that are subject to spread by electrostatic dispersal.

In specialized laboratories, the intentional, controlled generation of aerosols from toxin solutions may be undertaken to test antidotes or vaccines in experimental animals. These are extremely hazardous operations that should only be conducted after extensive validation of equipment and personnel, using non-toxic simulants. Aerosol exposure of animals should be done in a certified Class III BSC or hoodline. While removing exposed animals from the hoodline, and for required animal handling during the first 24 h after exposure, workers should take additional precautions, including wearing protective clothing (e.g., disposable Tyvek suit) and appropriate respiratory protection. To minimize the risk of dry toxin generating a secondary aerosol, areas of animal skin or fur exposed to aerosols should be gently wiped with a damp cloth containing water or buffered cleaning solution before the animals are returned to holding areas.

For high-risk operations involving dry forms of toxins, intentional aerosol formation, or the use of hollow-bore needles in conjunction with amounts of toxin estimated to be lethal for humans, consideration should be given to requiring the presence of at least two knowledgeable individuals at all times in the laboratory.⁷

Decontamination and Spills

Toxin stability varies considerably outside of physiological conditions depending upon the temperature, pH, ionic strength, availability of co-factors and other characteristics of the surrounding matrix. Literature values for dry heat inactivation of toxins can be misleading due to variations in experimental conditions, matrix composition, and experimental criteria for assessing toxin activity. Moreover, inactivation is not always a linear function of heating time; some protein toxins possess a capacity to re-fold and partially reverse

inactivation caused by heating. In addition, the conditions for denaturing toxins in aqueous solutions are not necessarily applicable for inactivating dry, powdered toxin preparations.

General guidelines for laboratory decontamination of selected toxins are summarized in Tables 1 and 2, but inactivation procedures should not be assumed to be 100% effective without validation using specific toxin bioassays. Many toxins are susceptible to inactivation with dilute sodium hydroxide (NaOH) at concentrations of 0.1-0.25N, and/or sodium hypochlorite (NaOCl) bleach solutions at concentrations of 0.1-0.5% (w/v). Use freshly prepared bleach solutions for decontamination; undiluted, commercially available bleach solutions typically contain 3-6% (w/v) NaOCl.

Depending upon the toxin, contaminated materials and toxin waste solutions can be inactivated by incineration or extensive autoclaving, or by soaking in suitable decontamination solutions (Table 2). All disposable material used for toxin work should be placed in secondary containers, autoclaved and disposed of as toxic waste. Contaminated or potentially contaminated protective clothing and equipment should be decontaminated using suitable chemical methods or autoclaving before removal from the laboratory for disposal, cleaning or repair. If decontamination is impracticable, materials should be disposed of as toxic waste.

In the event of a spill, avoid splashes or generating aerosols during cleanup by covering the spill with paper towels or other disposable, absorbent material. Apply an appropriate decontamination solution to the spill, beginning at the perimeter and working towards the center, and allow sufficient contact time to completely inactivate the toxin (Table 2).

Decontamination of buildings or offices containing sensitive equipment or documents poses special challenges. Large-scale decontamination is not covered explicitly here, but careful extrapolation from the basic principles may inform more extensive clean-up efforts.

Select Agent Toxins

Due diligence should be taken in shipment or storage of any amount of toxin. There are specific regulatory requirements for working with toxins designated as a "Select Agent" by the DHHS and/or the USDA. Select agents require registration with CDC and/or USDA for possession, use, storage and/or transfer. Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of the agent may require a permit from USDA/ APHIS/VS. A DoC permit may be required for the export of the agent to another country. See Appendix C for additional information.

Table 1. Physical Inactivation of Selected Toxins

Toxin	Steam Autoclave	Dry Heat (10 min)	Freeze-thaw	Gamma Irradiation
Botulinum neurotoxin	Yes ^a	> 100° C ^b	No ^c	Incomplete ^d
Staphylococcal Enterotoxin	Yes ^e	> 100° C; refolds ^f	No ^g	Incomplete ^h
Ricin	Yes ⁱ	> 100° C ⁱ	No ^j	Incomplete ^k
Microcystin	No ^l	> 260° C ^m	No ⁿ	ND
Saxitoxin	No ^l	> 260° C ^m	No ⁿ	ND
Palytoxin	No ^l	> 260° C ^m	No ⁿ	ND
Tetrodotoxin	No ^l	> 260° C ^m	No ⁿ	ND
T-2 mycotoxin	No ^l	> 815° C ^m	No ⁿ	ND
Brevetoxin (PbTx-2)	No ^l	> 815° C ^m	No ⁿ	ND

Notes:

ND indicates "not determined" from available decontamination literature.

- ^a Steam autoclaving should be at >121°C for 1 h. For volumes larger than 1 liter, especially those containing *Clostridium botulinum* spores, autoclave at >121°C for 2 h to ensure that sufficient heat has penetrated to kill all spores.^{8,9}
- ^b Exposure to 100°C for 10 min. inactivates BoNT. Heat denaturation of BoNT as a function of time is biphasic with most of the activity destroyed relatively rapidly, but with some residual toxin (e.g., 1-5%) inactivated much more slowly.¹⁰
- ^c Measured using BoNT serotype A at -20°C in food matrices at pH 4.1 – 6.2 over a period of 180 days.¹¹
- ^d Measured using BoNT serotypes A and B with gamma irradiation from a ⁶⁰Co source.^{12,13}
- ^e Protracted steam autoclaving, similar to that described for BoNT, followed by incineration is recommended for disposal of SE-contaminated materials.
- ^f Inactivation may not be complete depending upon the extent of toxin re-folding after denaturation. Biological activity of SE can be retained despite heat and pressure treatment routinely used in canned food product processing.¹⁴
- ^g SE toxins are resistant to degradation from freezing, chilling or storage at ambient temperature.¹⁵ Active SEB in the freeze-dried state can be stored for years.
- ^h References ^{15,16}
- ⁱ Dry heat of >100°C for 60 min in an ashing oven or steam autoclave treatment at >121°C for 1 h reduced the activity of pure ricin by >99%.¹⁷ Heat inactivation of impure toxin preparations (e.g., crude ricin plant extracts) may vary. Heat-denatured ricin can undergo limited refolding (<1%) to yield active toxin.
- ^j Ricin holotoxin is not inactivated significantly by freezing, chilling or storage at ambient temperature. In the liquid state with a preservative (sodium azide), ricin can be stored at 4°C for years with little loss in potency.
- ^k Irradiation causes a dose-dependent loss of activity for aqueous solutions of ricin, but complete inactivation is difficult to achieve; 75 MRad reduced activity 90%, but complete inactivation was not achieved even at 100 MRad.¹⁸ Gamma irradiation from a laboratory ⁶⁰Co source can be used to partially inactivate aqueous solutions of ricin, but dried ricin powders are significantly resistant to inactivation by this method.
- ^l Autoclaving with 17 lb pressure (121-132° C) for 30 min failed to inactivate LMW toxins.^{17,19} All burnable waste from LMW toxins should be incinerated at temperatures in excess of 815°C (1,500° F).
- ^m Toxin solutions were dried at 150° C in a crucible, placed in an ashing oven at various temperatures for either 10 or 30 min, reconstituted and tested for concentration and/or activity; tabulated values are temperatures exceeding those required to achieve 99% toxin inactivation.¹⁷
- ⁿ LMW toxins are generally very resistant to temperature fluctuations and can be stored in the freeze-dried state for years and retain toxicity.

Table 2. Chemical Inactivation of Selected Toxins

Toxin	NaOCl (30 min)	NaOH (30 min)	NaOCl + NaOH (30 min)	Ozone Treatment
Botulinum neurotoxin	> 0.1% ^a	> 0/25 N	ND	Yes ^b
Staphylococcal Enterotoxin	> 0.5% ^c	> 0.25 N	ND	ND
Ricin	> 1.0% ^d	ND	> 0.1% + 0.25N ^e	ND
Saxitoxin	≥ 0.1% ^e	ND	0.25% + 0.25N ^e	ND
Palytoxin	≥ 0.1% ^e	ND	0.25% + 0.25N ^e	ND
Microcystin	≥ 0.5% ^e	ND	0.25% + 0.25N ^e	ND
Tetrodotoxin	≥ 0.5% ^e	ND	0.25% + 0.25N ^e	ND
T-2 mycotoxin	≥ 2.5% ^{e,f}	ND	0.25% + 0.25N ^e	ND
Brevetoxin (PbTx-2)	≥ 2.5% ^{e,f}	ND	0.25% + 0.25N ^e	ND

Notes:

ND indicates "not determined" from available decontamination literature.

^a Solutions of NaOCl (#0.1%) or NaOH (> 0.25 N) for 30 min inactivate BoNT and are recommended for decontaminating work surfaces and spills of *C. botulinum* or BoNT. Chlorine at a concentration of 0.3-0.5 mg/L as a solution of hypochlorite rapidly inactivates BoNT (serotypes B or E tested) in water.²⁰ Chlorine dioxide inactivates BoNT, but chloramine is less effective.²¹

^b Ozone (> 2 mg/L) or powdered activated charcoal treatment also completely inactivate BoNT (serotypes A, B tested) in water under defined condition.^{20,22}

^c SEB is inactivated with 0.5% hypochlorite for 10-15 mi.²³

^d Ricin is inactivated by a 30 min exposure to concentrations of NaOCl ranging from 0.1-2.5%, or by a mixture of 0.25% NaOCl plus 0.25 N NaOH.¹⁷ In general, solutions of 1.0% NaOCl are effective for decontamination of ricin from laboratory surfaces, equipment, animal cages, or small spills.

^e The minimal effective concentration of NaOCl was dependent on toxin and contact time; all LMW toxins tested were inactivated at least 99% by treatment with 2.5% NaOCl, or with a combination of 0.25% NaOCl and 0.25N NaOH.¹⁷

^f For T-2 mycotoxin and brevetoxin, liquid samples, accidental spills, and nonburnable waste should be soaked in 2.5% NaOCl with 0.25% N NaOH for 4 h. Cages and bedding from animals exposed to T-2 mycotoxin or brevetoxin should be treated with 0.25% NaOCl and 0.025 N NaOH for 4 h. Exposure for 30 min to 1.0% NaOCl is an effective procedure for the laboratory (working solutions, equipment, animal cages, working area and spills) for the inactivation of saxitoxin or tetrodotoxin.

Decontamination of equipment and waste contaminated with select brevetoxins has been reviewed.¹⁹

Alternate methods of chemical decontamination: 1 N sulfuric or hydrochloric acid did not inactivate T-2 mycotoxin and only partially inactivated microcystin-LR, saxitoxin, and brevetoxin (PbTx-2). Tetrodotoxin and palytoxin were inactivated by hydrochloric acid, but only at relatively high molar concentrations. T2 was not inactivated by exposure to 18% formaldehyde plus methanol (16 h), 90% freon-113 + 10% acetic acid, calcium hypochlorite, sodium bisulfate, or mild oxidizing.¹⁷ Hydrogen peroxide was ineffective in inactivating T-2 mycotoxin. This agent did cause some inactivation of saxitoxin and tetrodotoxin, but required a 16 h contact time in the presence of ultraviolet light.

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Appendix J—NIH Oversight of Research Involving Recombinant Biosafety Issues

The NIH locus for oversight of recombinant DNA research is the Office of Biotechnology Activities (OBA), which is located within the Office of Science Policy, in the Office of the Director of the NIH. The OBA implements and manages the various oversight tools and information resources that NIH uses to promote the science, safety and ethics of recombinant DNA research. The key tools of biosafety oversight are the *NIH Guidelines*, IBCs, and the Recombinant DNA Advisory Committee (RAC). The NIH also undertakes special initiatives to promote the analysis and dissemination of information key to our understanding of recombinant DNA, including human gene transfer research. These initiatives include a query-capable database and conferences and symposia on timely scientific, safety, and policy issues. The NIH system of oversight is predicated on ethical and scientific responsibilities, with goals to promote the exchange of important scientific information, enable high-quality research, and help advance all fields of science employing recombinant DNA.

The *NIH Guidelines* promote safe conduct of research involving recombinant DNA by specifying appropriate biosafety practices and procedures for research involving the construction and handling of either recombinant DNA molecules or organisms and viruses that contain recombinant DNA. Recombinant DNA molecules are defined in the *NIH Guidelines* as those constructed outside of a living cell by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell. The *NIH Guidelines* are applicable to all recombinant DNA work at an institution that receives any funding from the NIH for recombinant DNA research. Compliance with the *NIH Guidelines* is mandatory for investigators conducting recombinant DNA research funded by the NIH or performed at or sponsored by any public or private entity that receives any NIH funding for recombinant DNA research. This broad reach of the *NIH Guidelines* is intended to instill biosafety practices throughout the institution, which is necessary if the practices are to be effective.

The *NIH Guidelines* were first published in 1976 and are revised as technological, scientific, and policy developments warrant. They outline the roles and responsibilities of various entities associated with recombinant DNA research, including institutions, investigators, biological safety officers, and the NIH (see Section IV of the *NIH Guidelines*). They describe four levels of biosafety and containment practices that correspond to the potential risk of experimentation and require different levels of review for recombinant DNA research, based on the nature and risks of the activity. These include:

1. Review by the RAC, and approval by the NIH Director and the IBC.
2. Review by the NIH OBA and approval by the IBC.

3. Review by the RAC and approvals by the IBC and Institutional Review Board.
4. Approval by the IBC prior to initiation of the research.
5. Notification of the IBC simultaneous with initiation of the work.

See Section III of the *NIH Guidelines* for additional details. In all instances, it is important to note that review by an IBC is required.

The federally mandated responsibilities for an IBC are articulated solely in the *NIH Guidelines*. Their membership, procedures, and functions are outlined in Section IV-B-2. Institutions, ultimately responsible for the effectiveness of IBCs, may define additional roles and responsibilities for these committees in addition to those specified in the Guidelines. To access the NIH Guidelines see the following Web site: http://oba.od.nih.gov/rdna/nih_guidelines_oba.html.

The Recombinant DNA Advisory Committee is a panel of national experts in various fields of science, medicine, genetics, and ethics. It includes individuals who represent patient perspectives. The RAC considers the current state of knowledge and technology regarding recombinant DNA research and advises the NIH Director and OBA on basic and clinical research involving recombinant DNA and on the need for changes to the *NIH Guidelines*.

Additional information on OBA, the *NIH Guidelines*, and the NIH RAC can be found at: <http://oba.od.nih.gov>.

Appendix K—Resources

Resources for information, consultation, and advice on biohazard control, decontamination procedures, and other aspects of laboratory and animal safety management include:

AAALAC International

Association for Assessment and Accreditation
of Laboratory Animal Care International
5283 Corporate Drive
Suite 203
Fredrick, MD 21703-2879
Telephone: (301) 696-9626
Fax: (301) 696-9627
Web site: <http://www.aaalac.org>

American Biological Safety Association (ABSA)

American Biological Safety Association
1200 Allanson Road
Mundelein, IL 60060-3808
Telephone: (847) 949-1517
Fax: (847) 566-4580
Web site: <http://www.absa.org>

CDC Etiologic Agent Import Permit Program

Centers for Disease Control and Prevention
Etiologic Agent Import Permit Program
1600 Clifton Road, NE Mailstop: F-46
Atlanta, GA 30333
Telephone: (404) 718-2077
Fax: (404) 718-2093
Web site: <http://www.cdc.gov/od/eaipp>

CDC Office of Health and Safety

Centers for Disease Control and Prevention
Office of Health and Safety
Mailstop: F-05
1600 Clifton Road, NE
Atlanta, GA 30333
Telephone: (404) 639-7233
Fax: (404) 639-2294
Web site: www.cdc.gov/biosafety

CDC Select Agent Program

Centers for Disease Control and Prevention
Division of Select Agents and Toxins
Mailstop: A-46
1600 Clifton Road, NE
Atlanta, GA 30333
Telephone: (404) 718-2000
Fax: (404) 718-2096
Web site: www.selectagents.gov

Clinical and Laboratory Standards Institute

940 West Valley Road, Suite 1400
Wayne, PA 19087
Telephone: (610) 688-0100
Fax: (610) 688-0700
Web site: <http://www.clsi.org>

College of American Pathologists

1350 I St. N.W. Suite 590
Washington, DC 20005-3305
Telephone: (800) 392-9994
Telephone: (202) 354-7100
Fax: (202) 354-7155
Web site: <http://www.cap.org>

Department of the Army

Biological Defense Safety Program
Department of Defense
32 CFR Parts 626, 627
Web site: www.gpo.gov

National B-Virus Resource Laboratory

National B Virus Resource Laboratory
Attention: Dr. Julia Hillard
Viral Immunology Center
Georgia State University
50 Decatur Street
Atlanta, GA 30303
Telephone: (404) 651-0808
Fax: (404) 651-0814
Web site: <http://www2.gsu.edu/~wwwvir>

NIH Division of Occupational Health and Safety

National Institutes of Health
Division of Occupational Health and Safety
Building 13, Room 3K04
13 South Drive, MSC 5760
Bethesda, MD 20892
Telephone: (301) 496-2960
Fax: (301) 402-0313
Web site: <http://dohs.ors.od.nih.gov/index.htm>

NIH Office of Biotechnology Activities

National Institutes of Health
Office of Biotechnology Activities
6705 Rockledge Drive
Suite 750, MSC 7985
Bethesda, MD 20892
Telephone: (301) 496-9838
Fax: (301) 496-9839
Web site: <http://oba.od.nih.gov>

NIH Office of Laboratory Animal Welfare (OLAW)

National Institutes of Health
Office of Laboratory Animal Welfare (OLAW)
RKL 1, Suite 360, MSC 7982
6705 Rockledge Drive
Bethesda, MD 20892-7982
Telephone: (301) 496-7163
Web site: <http://grants.nih.gov/grants/olaw/olaw.htm>

Occupational Safety and Health Administration

U.S. Department of Labor
200 Constitution Avenue NW
Washington, DC 20210
Telephone: (800) 321-6742
Web site: <http://www.osha.gov/index.html>

USDA-APHIS National Center for Import / Export

USDA Animal and Plant Health Inspection Service Veterinary Services National
Center for Import and Export
4700 River Road, Unit 40
Riverdale, MD 20737
Web site: http://www.aphis.usda.gov/import_export/index.shtml

USDA Agriculture Select Agent Program

USDA Agriculture Select Agent Program
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
4700 River Road, Unit 2, Mailstop 22
Riverdale, MD 20737
Web site: http://www.aphis.usda.gov/programs/ag_selectagent/index.shtml

USDA National Animal Disease Center

U.S. Department of Agriculture
National Animal Disease Center
P.O. Box 70
2300 Dayton Road
Ames, IA 50010
Telephone: (515) 663-7200
Fax: (515) 663-7458
Web site: http://www.ars.usda.gov/main/site_main.htm?modecode=36-25-30-00

USDA Plant Select Agents & Plant Protection and Quarantine

Animal and Plant Health Inspection Service
Plant Protection and Quarantine, Permits, Agricultural Bioterrorism
U.S. Department of Agriculture
4700 River Road, Unit 133
Riverdale, MD 20737
Telephone: (877) 770-5990
Fax: (301) 734-5786
Web site: http://www.aphis.usda.gov/programs/ag_selectagent/index.shtml;
and <http://www.aphis.usda.gov/permits/index.shtml>

US Department of Transportation

Hazardous Materials Center
Pipeline & Hazardous Materials Center
U.S. Department of Transportation
400 7th Street, S.W.
Washington, DC 20590
Telephone: (800) 467-4922
Web site: <http://www.phmsa.dot.gov/hazmat>

US Department of Commerce

Export Administration Program
Bureau of Industry and Security (BIS)
Export Administration Regulations (EAR)
U.S. Department of Commerce
14th Street and Constitution Avenue, N.W.
Washington, DC 20230
Telephone: (202) 482-4811
Web site: <http://www.access.gpo.gov/bis/index.html>

World Health Organization Biosafety Program

World Health Organization Biosafety Program
Avenue Appia 20
1211 Geneva 27
Switzerland
Telephone: (+ 41 22) 791 21 11
Fax: (+ 41 22) 791 3111
Web site: <http://www.who.int/ihr/biosafety/en>

Appendix L—Acronyms

A1HV-1	Alcelaphine Herpesvirus-1
ABSA	American Biological Safety Association
ABSL	Animal Biosafety Level
ACAV	American Committee on Arthropod-Borne Viruses
ACIP	Advisory Committee on Immunization Practices
ACG	Arthropod Containment Guidelines
ACL	Arthropod Containment Levels
ACME	American Committee of Medical Entomology
AHS	African Horse Sickness
AHSV	African Horse Sickness Virus
AKAV	Akabane Virus
APHIS	Animal and Plant Health Inspection Service
APMV-1	Avian Paramyxovirus Type 1
ASF	African Swine Fever
ASFV	African Swine Fever Virus
ASHRAE	American Society of Heating, Refrigerating, and Air-Conditioning Engineers
ASTMH	American Society of Tropical Medicine and Hygiene
BCG	Bacillus Calmette-Guérin
BDV	Border Disease Virus
BMBL	Biosafety in Microbiological and Biomedical Laboratories
BoNT	Botulinium neurotoxin
BSC	Biological Safety Cabinet
BSE	Bovine Spongiform Encephalopathy
BSL	Biosafety Level
BSL-3-Ag	BSL-3-Agriculture
BSO	Biological Safety Officer
BTV	Bluetongue Virus
BVDL	Bovine Viral Diarrhea Virus
CAV	Constant Air Volume
CBPP	Contagious Bovine Pleuropneumonia
CCPP	Contagious Caprine Pleuropneumonia
CETBE	Central European Tick-Borne Encephalitis
CDC	Centers for Disease Control and Prevention
CHV-1	Cercopithecine Herpesvirus-1
CJD	Creutzfeldt-Jakob Disease
CJIS	Criminal Justice Information Services Division
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
CSFV	Classical Swine Fever Virus
DHHS	Department of Health and Human Services
DoC	Department of Commerce
DOD	Department of Defense
DOL	Department of Labor

DOT	Department of Transportation
EBV	Epstein-Barr Virus
EEE	Eastern Equine Encephalomyelitis
EPA	Environmental Protection Agency
EtOH	Ethanol
FDA	Food and Drug Administration
FFI	Fatal Familial Insomnia
FMD	Foot and Mouth Disease
FMDV	Foot and Mouth Disease Virus
GI	Gastrointestinal Tract
GSS	Gerstmann-Straussler-Scheinker Syndrome
HEPA	High Efficiency Particulate Air
HBV	Hepatitis B Virus
HCMV	Human Cytomegalovirus
HCV	Hepatitis C Virus
HD	Heartwater Disease
HDV	Hepatitis D Virus
HFRS	Hemorrhagic Fever with Renal Syndrome
HHV	Human Herpes Virus
HHV-6A	Human Herpes Virus -6A
HHV-6B	Human Herpes Virus -6B
HHV-7	Human Herpes Virus -7
HHV-8	Human Herpes Virus -8
HIV	Human Immunodeficiency Virus
HPAI	Highly Pathogenic Avian Influenza
HPAIV	Highly Pathogenic Avian Influenza Virus
HPS	Hantavirus Pulmonary Syndrome
HSV-1	Herpes Simplex Virus-1
HSV-2	Herpes Simplex Virus-2
HTLV	Human T-Lymphotropic Viruses
HVAC	Heating, Ventilation, and Air Conditioning
IACUC	Institutional Animal Care and Use Committee
IATA	International Air Transport Association
IBC	Institutional Biosafety Committee
ICAO	International Civil Aviation Organization
ID	Infectious Dose
ID ₅₀	Number of organisms necessary to infect 50% of a group of animals
IgG	Immunoglobulin
ILAR	Institute for Laboratory Animal Research
IND	Investigational New Drug
IPM	Integrated Pest Management
IPV	Inactivated Poliovirus Vaccine
ISA	Infectious Salmon Anemia

ISAV	Infectious Salmon Anemia Virus
LAI	Laboratory-Associated Infections
LCM	Lymphocytic Choriomeningitis
LCMV	Lymphocytic Choriomeningitis Virus
LD	Lethal Dose
lfm	Linear Feet Per Minute
LGV	Lymphogranuloma Venereum
LMW	Low Molecular Weight
LSD	Lumpy Skin Disease
LSDV	Lumpy Skin Disease Virus
MCF	Malignant Catarrhal Fever
MenV	Menangle Virus
MMWR	Morbidity and Mortality Weekly Report
MPPS	Most Penetrating Particle Size
NaOCl	Sodium Hypochlorite
NaOH	Sodium Hydroxide
NBL	National Biocontainment Laboratory
NCI	National Cancer Institute
ND	Newcastle Disease
NDV	Newcastle Disease Virus
NHP	Nonhuman Primate
NIH	National Institutes of Health
NIOSH	National Institute for Occupational Safety and Health
OBA	NIH Office of Biotechnology Activities
OIE	World Organization for Animal Health
OPV	Oral Poliovirus Vaccine
OSHA	Occupational Safety and Health Administration
PAPR	Positive Air-Purifying Respirator
PBT	Pentavalent Botulinum Toxoid Vaccine
PPD	Purified Protein Derivative
PPM	Parts Per Million
PPRV	Pest des Petits Ruminants Virus
Prp	Prion Protein
RAC	Recombinant DNA Advisory Committee
RBL	Regional Biocontainment Laboratory
RP	Rinderpest
RPV	Rinderpest Virus
RVF	Rift Valley Fever
RVFV	Rift Valley Fever Virus
SALS	Subcommittee on Arbovirus Laboratory Safety
SARS	Severe Acute Respiratory Syndrome
SARS-CoV	SARS-Associated Coronavirus
SCID	Severe Combined Immune Deficient
SC type	Small-Colony type

SE	Staphylococcal Enterotoxins
SEA	SE Serotype A
SEB	SE Serotype B
SIV	Simian Immunodeficiency Virus
SGP	Sheep and Goat Pox
SGPV	Sheep and Goat Pox Virus
SOP	Standard Operating Procedure
SVCV	Spring Viremia of Carp Virus
SVD	Swine Vesicular Disease
SVDV	Swine Vesicular Disease Virus
TLV	Threshold Limit Values
TME	Transmissible Mink Encephalopathy
TSE	Transmissible Spongiform Encephalopathy
UV	Ultraviolet
USAMRIID	U.S. Army Medical Research Institute of Infectious Diseases
USDA	U.S. Department of Agriculture
USPS	United States Postal Service
UPS	Uninterrupted Power Supply
VAV	Variable Air Volume
VEE	Venezuelan Equine Encephalitis
VS	Veterinary Services
VZV	Varicella-Zoster Virus
WEE	Western Equine Encephalomyelitis
WHO	World Health Organization
WNV	West Nile Virus

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