University of California, Merced
Environmental Health and Safety

Biosafety Manual

Revised October 2015

University of California, Merced

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DEFINITION OF "BIOHAZARDOUS MATERIAL"

"Biohazardous Materials" shall be defined as:

- All infectious organisms (bacteria, fungi, parasites, Prions, rickettsia, viruses, yeasts, etc.) which can cause disease in humans, or cause significant environmental or agricultural impact.
- Includes work with human or primate tissues, fluids, cells, or cell culture, recombinant nucleic acid molecules, synthetic nucleic acid molecules, synthesis of transgenic plants or animals, human gene therapy, releases of recombinant DNA to the environment, work with non-indigenous plants or animals, and work with animals (vertebrate or invertebrate) known to be infected with a zoonotic disease.

The University has established the Institutional Biosafety Committee (IBC) under the guidelines of the NIH Office of Biotechnology Activities to determine on a case-by-case basis whether an experiment utilizes biohazardous material, and regulate use of this biohazardous material by researchers or teaching labs affiliated with the University of California, Merced, whether the work is conducted on campus or at an approved off-campus location.

POLICIES AND RESPONSIBILITIES

A. Campus Policy

1. Biological agents in research shall be used in a manner that will not adversely affect the health, safety, or well-being of faculty, staff, students, campus visitors, neighboring human populations, wild and domestic animals or the environment.

2. The U.S. Public Health Service publication *Biosafety in Microbiological and Biomedical Laboratories 5th Edition* (http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf) has been adopted, with some revisions, as the campus standard for the use of biological agents.

3. A Biological Use Authorization (BUA) is required for research involving recombinant nucleic acids, synthetic nucleic acid molecules, infectious agents, toxins, transgenic animals, BSL 2 organisms, human gene transfer, or human, sheep or Old World primates or their source material. Application forms are available on the Office of Environmental Health and Safety (EH&S) web site http://ehs.ucmerced.edu/. Activities requiring a BUA must be approved by the Biosafety Officer and the Institutional Biosafety Committee (IBC) chair if no recombinant nucleic acid or synthetic nucleic acid molecule work is involved. Experiments using recombinant nucleic acid or synthetic nucleic acid molecule must be approved by a quorum of the IBC. No research involving Biosafety Level 4 materials is permitted at UC Merced.

4. Newly isolated or recognized infectious agents of unknown pathogenicity shall be treated as Biosafety Level 2 or greater infectious agents, and those with evidence of oncogenicity shall be treated as prescribed by the guidelines of the National Cancer Institute:

    a. Low-risk, oncogenic viruses will be treated as Biosafety Level 2.
    b. Moderate-risk, oncogenic viruses will be treated as Biosafety Level 3. Currently no BSL-3 work is conducted on the UC Merced Campus.
    c. High-risk, oncogenic viruses will be treated as Biosafety Level 4. Currently BSL-4 work is prohibited on the UC Merced campus.

5. All incoming materials containing aerosol transmissible pathogens (ATPs) will be treated as containing virulent or wild-type pathogen until procedures at the laboratory have verified the pathogen is attenuated or deactivated. Select agents and toxins require special registration with

the Centers for Disease Control and Prevention (CDC). Contact EH&S for registration information (Appendix B).

6. All research involving recombinant nucleic acids and synthetic nucleic acid molecules shall be treated as prescribed by the most recent edition of NIH's *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* and as prescribed by law.

    a. Projects involving recombinant and synthetic nucleic acid molecules that are listed as "exempt" by the NIH Guidelines require registration with the IBC as necessary.

    b. Projects that are not exempt must be approved by the IBC.

7. Activities involving the release of plants or organisms into the environment require a completed USDA permit application. Before submitting the application to the USDA, the IBC must review and approve it.

8. The Campus Biosafety Manual will be the basis for general biosafety guidelines in the laboratory. Laboratory personnel will be expected to follow practices outlined in this manual as well as the prudent practices specific to the project(s) in which they have been involved.

B. Responsibilities

1. Laboratory staff, students and postdoctoral fellows who work in the laboratory are responsible for the following:

    a. Being familiar with all protocols and organisms used in the laboratory regardless of whether or not they work directly with them.

    b. Knowing all emergency procedures established by the Principal Investigator (PI).

    c. Completing training and verifying documentation of appropriate training.

    d. Following all appropriate laboratory practices as outlined in this manual and all additional practices outlined in the Laboratory Safety Plan Supplement.

2. The PI of a research project or teaching laboratory is responsible for the following:

    a. Developing specific protocols to ensure the safe use of infectious agents.

    b. Developing specific protocols that outline proper emergency procedures in the case of an accidental exposure of personnel or the environment to the biological agents.

    c. Completing the application for Biological Use Authorization form and providing specific safety protocol information by following instructions set forth in this manual. Forms are available on the EH&S website http://ehs.ucmerced.edu/. No work may commence until the project has been approved by the IBC for recombinant nucleic acids and synthetic nucleic acid molecules work, or the IBC chair and Biosafety Officer for work at BSL 1 or above.

    d. Obtaining approval from the different committees relevant to the project. For example, obtaining approval of an Animal Use and Care Protocol if the project involves animals.

e. Obtaining the required approvals from the appropriate Dean.

f. Complying with the safety protocol, this manual, campus policy, and any applicable federal and state laws and regulations.

g. Training all personnel involved in the project so that they have a complete understanding of the hazards involved, safety procedures required and the emergency protocols in place. This includes animal care personnel not directly supervised by the PI, who provide care for infected animals. Documentation of training must be kept on file.

h. Providing the proper Personal Protective Equipment for each laboratory member or visitor, and replacing this equipment as it is damaged or no longer provides appropriate protection.

i. Complying with medical waste laws by maintaining a designated medical waste autoclave or an approved medical waste accumulation area as well as handling medical waste in compliance with the Medical Waste Management Act.

j. Notifying the IBC of any changes in personnel, procedures, or protocols; or filing an annual report indicating the work has not changed.

k. Monitoring the access of laboratory visitors and ensuring their safety.

l. Performing an annual self-audit of the laboratory.

3. The Dean is responsible for the following:

a. Ensuring the health and safety of employees, visitors, students and postdoctoral fellows while in UC Merced facilities under departmental control.

b. Authorizing biological agent research and use of safety protocols prior to the start of work on the project within the department.

c. Signing copies of the BUA and safety protocols certifying your approval prior to submittal to the IBC.

d. Ensuring departmental compliance with applicable laws, regulations and guidelines covering the use of biological agents in research.

4. Environmental Health & Safety is responsible for the following:

a. Providing consultation in the development of safety protocols as requested by the PI or department chair.

b. Advising generators on proper waste treatment and disposal methods in accordance with federal, state and campus standards.

c. Reviewing all applications for the use of biological agents and preparing recommendations for the IBC.

d. Ensuring department and user compliance with the IBC's recommendations.

e. Scheduling and performing annual inspections of facilities.

f. Monitoring completion of projects through annual updates of protocols.

g. Keeping records and copies of applicable laws and regulations.

h. Providing training materials and classes.

i. Providing application materials upon request.

5. The Institutional Biosafety Committee (IBC) is responsible for the following:

a. Ensuring the safe use of biological agents.

b. Assessing compliance with this manual, which includes determining whether a project involving biological materials is exempt from registration or must be registered and approved by the IBC.

c. Recommending acceptance or rejection of all proposed projects requiring authorization or registration.

d. Requesting NIH/OBA determination of select experiments falling under Section III-A-1-a that may require RAC review and NIH Director approval.

e. Formulating and recommending changes in campus policy for the safe use of biological agents and complying with federal and state laws, regulations and guidance standards.

f. Authorizing EH&S to terminate or curtail any project or any teaching program involving the use of biological agents when it is in the best interest of the health and safety of the campus community.

g. Establishing the level of medical surveillance for each program after reviewing the recommendation of the campus Occupational Health physician.

BIOHAZARD USE APPLICATION PROCESS & GENERAL INFORMATION

A. Requirements for Approval of Projects Using Biohazardous Agents

Biological agents classified according to their Biosafety Level are listed in Appendix A. If the agent of concern is not listed, contact EH&S. BUAs are required for all research involving recombinant nucleic acids, synthetic nucleic acid molecules, infectious agents, toxins, transgenic animals, BSL 2 work or above, human gene transfer, or human, sheep, or Old World primates or their source; application forms are available on the EH&S web site.

1. Biosafety Level (BSL)1 and Animal Biosafety Level (ABSL)1
   BSL 1 and ABSL1 biohazardous agents are defined as organisms that are not known to cause disease in healthy human adults. Some Biosafety Level 1 work requires BUA approval by the Biosafety Officer and IBC Chair. Please call 228-4639 with any questions.

2. Biosafety Level (BSL) 2 or 3 and Animal Biosafety Level (ABSL) 2 or 3

a. All projects involving biohazardous agents classified as BSL 2 or 3 must be reviewed and approved by the chair of the IBC or the chair's designee and the Biosafety Officer and must follow the general guidelines specified for that level.

b. Any recombinant nucleic acid or synthetic nucleic acid molecule work will be approved by the entire IBC.

c. The committee may impose additional requirements after a full review of the Biological Use Authorization application. Conditional approval may be given to applications that have been reviewed and require only minor changes. Full approval is granted once all changes have been completed. Under certain circumstances pursuant to Section III-A-1-a of the NIH Guidelines, the IBC may refer an experiment proposed by BUA to the NIH/OBA for RAC review and NIH Director approval. A BUA with full approval is good for three years before it requires renewal; annually a letter must be sent to the committee indicating if the nature of the work has changed or not.

d. Biosafety levels and/or animal biosafety levels are listed in Appendix A, "Biosafety Levels for Infectious Agents and Infected Animals".

e. Other committee approvals relevant to the project must also be obtained before work commences.

3. Select agents require special registration with the Centers for Disease Control and Prevention (CDC) (see Appendix B). Contact EH&S for further information and registration procedures.

4. Projects involving Biosafety Level 4 and Animal Biosafety Level 4 organisms are prohibited at UC Merced.

B. Requirements for Approval of recombinant or synthetic Nucleic Acid Projects

Classification and containment requirements for use of recombinant DNA molecules can be found in the latest edition of NIH's *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*. Copies can be obtained from CDC/NIH, contact EH&S for assistance.

All projects involving recombinant nucleic acids and synthetic nucleic acid molecules **must** be reviewed and approved by the IBC and must follow the general procedures outlined in NIH's most recent *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*. Additional requirements may be imposed by the Committee after a Biological Use Authorization application is reviewed and approved. Other committee approvals relevant to your project must also be obtained before work commences. Projects involving BSL-4 organisms are prohibited at UC Merced.

C. Audits

Laboratory audits will be performed annually by EH&S. These audits are designed to verify that all laws and regulations outlined in this manual and by state and federal organizations are followed. This includes the use of adequate facilities and proper maintenance of those facilities. Additionally, interviews of laboratory personnel may be conducted to ensure that they are aware of proper safety and emergency protocols and that they are informed about the properties of the organisms with which they are working. Training records, waste disposal records and other documentation will be reviewed at this time.

Self-audits are to be performed annually by the laboratory following the EH&S self-audit guide and a completed and dated copy of the self-audit kept in the lab.

D. Annual Updates

After approval, BUAs are good for three years before renewal. However, PIs are required to submit an annual update in the form of a verification letter and data sheet. Verification letters and data sheets will be sent from EH&S to PIs for review and revision. The annual update will allow the PI to discontinue the protocol, verify that the protocol is still active or submit amendments to it. Significant amendment changes must be approved by the IBC before changes are allowed in the laboratory. The annual update will allow PIs to make minor changes to projects or add or delete personnel from their protocols. The update may also be necessary or helpful for approval of new or ongoing grants.

E. Training

1. Training is required for all employees, including students and volunteers, working in labs or animal rooms where biological agents are used. The PI, laboratory director or animal facility director is responsible for ensuring that adequate instruction is provided to all personnel who will have contact or will be involved with biological agents. **This includes training for specific tasks that employees will perform.** All training must be documented and the signed documents must be kept in the lab or with departmental records.

2. Personnel should not begin working with biological agents before the Biological Use Authorization application has been approved. Documentation of their training must be submitted with the application. Personnel must always receive training when new agents, procedures, processes and equipment are introduced. **They will be prohibited from performing new duties until they are properly trained.** Records of this training must also be kept on file and be available for review.

F. Medical Surveillance

1. **Immunizations**
   Using certain biohazardous agents may require immunizations for personnel. An Occupational Health physician may recommend immunization for personnel exposed, or potentially exposed, to certain biohazardous agents as a result of their participation in a project.

2. **Medical Examinations**
   An Occupational Health physician in consultation with the PI and the appropriate Dean may require medical examinations including collection of blood specimens for future analysis of personnel exposed, or potentially exposed, to certain biological agents. When appropriate, baseline serum samples from animal care and other at-risk personnel are collected and stored. Additional serum samples may be collected periodically depending on the agents handled or the function of the facility. The decision to establish a serologic surveillance program must take into account the availability of methods for the assessment of antibody to the agent(s) of concern. The program should provide for the testing of serum samples at each collection interval and the communication of results to the participants.

3. **Cost for Medical Surveillance**

The costs for medical surveillance will be charged to the appropriate school, research or instructional budget. Medical surveillance costs for employees and students should be planned before conducting the research project.

G. Health Service of Individuals Having Animal Contact

1. **Definition**
   The phrase "individuals having animal contact" refers to employees and students who, in the course of their employment, research or education, have substantial contact with research animals used for biohazardous agent studies (e.g., animal caretakers, animal technicians, veterinarians) and where such contact may pose a threat to humans or animals.

2. **Responsibility**
   It is the responsibility of the immediate supervisor to establish whether the animal contact is substantial or poses a possible health threat. This should be established with consultation of the campus IACUC office.

3. **Notification**
   The supervisors of individuals having animal contact will enroll those personnel in the online Occupational Health Surveillance System (OHSS).

H. Laws, Regulations and Guidelines

1. **Bloodborne Pathogens Regulation**
   The presence of bloodborne pathogens in the workplace is regulated by Tile 8 of the California Code of Regulations (CCR) Section 5193, Bloodborne Pathogens.

   a. Working with biohazardous agents may include working with human blood or OPIM, including human cell lines. If you are doing such work, the Bloodborne Pathogen (BBP) regulation may apply to your project. The BBP regulations and Biological Use Authorizations are different programs, but requirements and information may overlap and can be utilized in both programs.

   b. Cal-OSHA developed its BBP regulations (CCR, Title 8, Section 5193) in response to the federal government's published rule (29 CFR Part 1910.1030) governing occupational exposure to bloodborne pathogens. This standard provides guidelines to eliminate or minimize employee exposure to human bloodborne pathogens.

   c. The state standard requires the employer to have a written Exposure Control Plan (ECP) which identifies potential worker exposures and outlines measures to eliminate or minimize exposures, including training, Personal Protective Equipment (PPE), a hepatitis B vaccination program and engineering and work practice controls. A schedule and method of implementation must be included in the plan. At this point, the ECP and BUA may contain the same information.

   d. The standard applies to all campus employees in those job classifications that have potential for occupational exposure. The job classes affected include, but are not limited to, faculty, researchers, teaching assistants, laboratory technicians, medical personnel, first-aid providers, custodial staff, health and safety representatives and others with potential occupational exposure.

e. Contact EH&S for information on the campus Exposure Control Plan for compliance with the Cal-OSHA Bloodborne Pathogen Standard (CCR Title 8, Section 5193).

2. **Medical Waste Management Act**
The State Medical Waste Management Act (MWMA), California Health & Safety Code Division 20, Chapter 6.1 regulates the handling, storage, treatment and disposal of medical waste. Most activities involving biohazardous agents will involve medical-waste generation. Generators must register their facility as a medical waste generator and develop a specific medical waste management plan. The State Department of Health Services (DHS) must permit medical waste treatment facilities, including autoclaves and incinerators.

3. **Biological Safety Cabinets**
The certification, use and maintenance of Class II biological safety cabinets is described in the NSF International Standard 49. The use and maintenance of biological safety cabinets is regulated by Cal-OSHA in Title 8, CCR, Section 5154.2, Ventilation Requirements for Biosafety Cabinets. Cabinets used for work at BSL 2 or above must be certified annually.

4. **Biosafety Practices**
This biosafety manual is primarily based on the CDC/NIH guideline entitled Biosafety in Microbiological and Biomedical Laboratories.

5. **Biosafety Training**
Biosafety Training Biosafety Training is mandated by title 8, CCR, Section 3203, Injury and Illness Prevention Program, (IIPP) and Title 8, CCR, Section 5193, Bloodborne Pathogens.

6. **Recombinant or Synthetic Nucleic Acid Molecules**
Use of recombinant nucleic acids and synthetic nucleic acid molecules is governed by NIH's *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*.

I. Institutional Review Board

This committee will advise the Vice Chancellor of Research on research protocols related to behavioral, clinical and physiological studies involving human subjects. Please call the Office of Research at (209) 228-4429.

PRINCIPLES OF BIOSAFETY

A. Containment

The term "containment" is used in describing safe methods for managing infectious agents 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 people and the outside environment to potentially hazardous agents. The three elements of containment include **laboratory safety practices and techniques, safety equipment** and **facility design.**

Containment includes primary containment, the protection of personnel and the laboratory environment from exposure to biohazardous agents, and secondary containment, the protection

of the environment outside of the laboratory from exposure to biohazardous agents. Primary containment is possible through good microbiological techniques and the proper use of appropriate safety equipment. Secondary containment is provided by a combination of facility design and operational practices.

1. Laboratory Safety Practices and Techniques

    a. The most important element of containment is strict adherence to standard microbiological safety practices and techniques. Persons working with infectious agents or 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 PI or laboratory supervisor is responsible for providing or arranging for appropriate training of personnel.

    b. Each laboratory should develop Standard Operating Procedures (SOPs) that identify specific hazards that will or may be encountered as well as specific practices and procedures designed to minimize or eliminate risks. The SOPs should be included in your LSPS. Personnel should be advised of special hazards and should be required to read and to follow the required practices and procedures. A scientist trained and knowledgeable in appropriate laboratory techniques, safety procedures and hazards associated with the handling of infectious agents must direct laboratory activities.

    c. When standard laboratory practices are not sufficient to control the hazard associated with a particular agent or laboratory procedure, additional measures may be needed. The PI is responsible for selecting additional safety practices, which must be in keeping with the hazard associated with the biological agent or procedure.

    d. Laboratory personnel safety practices and techniques must be supplemented by appropriate facility design and engineering features, safety equipment and management practices.

2. Safety Equipment (Primary Barriers)

    a. Safety Equipment includes biological safety cabinets, enclosed containers and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The Biological Safety Cabinet (BSC) is the principal engineering control used to provide containment of infectious splashes or aerosols generated by many microbiological procedures.

    b. Safety equipment also may include items for personal protection such as protective clothing, respirators, face shields, safety glasses or goggles. Personal Protective Equipment (PPE) is often used when working with biohazardous agents. In some situations, personal protective clothing may form the primary barrier between personnel and the biohazardous agents.

    c. All laboratories are required to have completed the LHAT /HAT for identifying laboratory hazards, and keeping the names of laboratory personnel current. PPE required by this document must be used in the lab.

3. Facility Design (Secondary Barriers)

a. The design of a facility is important in providing a barrier to protect people working inside and outside the laboratory and to protect people or animals in the community from infectious agents that may be accidentally released in the laboratory. Facilities must be commensurate with the laboratory's function and the recommended biosafety level for the agent being manipulated.
b. 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 Biosafety Level 2 and 3 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.

c. As the risk for aerosol transmission increases, higher levels of primary containment and multiple secondary barriers may become necessary to prevent infectious agents from escaping into the environment. Such design features could include specialized ventilation systems to assure 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 for isolation of the laboratory.

B. Biosafety Levels

There are four biosafety levels that consist of combinations of laboratory safety practice and techniques, safety equipment and laboratory facilities. Each combination is specifically appropriate for the operations performed, for the documented or suspected routes of transmission of the infectious agents, and for the laboratory function or activity. The recommended biosafety level for an organism represents the conditions under which the agents can be ordinarily handled safely.

Biosafety Level 1

Biosafety Level 1 is appropriate for work done with defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans. It represents a basic level of containment that relies on standard microbiological practices. Primary barriers required are a lab coat, gloves, and safety glasses (goggles or face shield as needed), as well as a sink for hand washing.

Biosafety Level 2

Biosafety Level 2 is applicable to work done with a broad spectrum of indigenous, moderate-risk agents present in the community and associated with human disease of varying severity. Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures or ingestion of infectious materials. Primary barriers such as a lab coat, gloves and safety glasses or other appropriate eye/face protection should be used. Generally, a certified Biosafety Cabinet should be used for manipulations of cells, tissues, and other materials. Secondary barriers such as hand washing and waste decontamination facilities must be available.

Biosafety Level 3

Biosafety Level 3 is applicable to work done with indigenous or exotic agents with a potential for respiratory transmission and which may cause serious and potentially lethal infection. Primary hazards to personnel working with these agents (i.e., Mycobacterium tuberculosis, St. Louis encephalitis virus and Coxiella burnetii) include autoinoculation, ingestion and exposure to infectious aerosols. For example, all laboratory manipulations should be performed in biological

safety cabinets or other enclosed equipment. Secondary barriers include controlled access to the laboratory and a specialized ventilation system (e.g., HEPA filters, incinerators, etc.) that minimizes the release of infectious aerosols from the laboratory.

Biosafety Level 4

Biosafety Level 4 is applicable for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease that can be transmitted via the aerosol route and for which there is no available vaccine or therapy. All manipulations of potentially infected materials and isolates pose a high risk of exposure and infection to personnel, the community and the environment. The facility is a specially designed building with specialized ventilation and waste management systems to prevent release of viable agents to the environment. A Biosafety Level 4 laboratory or facility is prohibited at UC Merced.

C. Biosafety Level Criteria

TABLE 1 - SUMMARY OF RECOMMENDED BIOSAFETY LEVELS FOR INFECTIOUS AGENTS

Bio-Safety Level	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to cause disease in healthy adults.	Standard Microbiological Practices	Laboratory coats, gloves and safety glasses; other face/eye protection as needed	Open bench-top sink required
2	Associated with human disease, hazard = autoinoculation, ingestion, mucous membrane exposure	BSL-1 practice plus: - Limited access - Biohazard warning signs - Sharps precautions - Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers = Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials as well as PPE listed for BSL 1, plus face protection as needed	BSL-1 plus ensure that an autoclave is available
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences.	BSL-2 practice plus: - Controlled access - Decontamination of all waste - Decontamination of lab clothes before laundering - Baseline serum	Primary barriers = Class I or II BSCs or other physical containment devices used for all manipulations of agents as well as PPE such as protective lab clothing, gloves, safety glasses or other face/eye protection, and respiratory protection, as needed	BSL-2 plus: - Physical separation from access corridors - Self-closing, double door access - Exhaust air not recirculated - Negative airflow into laboratory
4	BSL4 work is not	permitted at UCM		

Adapted from the 5th Edition of the "Biosafety in Microbiological and Biomedical Laboratories" Handbook.

BIOLOGICAL SAFETY GUIDELINES

A. Biosafety Cabinets

Biosafety cabinets (BSCs) are used to provide primary containment in the laboratory when using potentially infectious materials and can be used for manipulation of sterile cultures. BSCs must be used in Biosafety Level 2 or 3 projects if aerosol-generating procedures are conducted, high concentrations of infectious agents are used, or if large volumes of infectious agents are used. There are three types of BSCs as defined by CDC/NIHs *Biosafety in Microbiological and Biomedical Laboratories*. **EH&S should be consulted for selection, purchase, installation and use of BSCs on campus.**

1. Testing and Certification of BSCs

   BSCs must be tested and certified annually or after installation, alterations, maintenance, or earthquakes. Testing and certification of BSCs will be performed by an outside contract and paid for by the laboratory; contact EH&S for more information. EH&S maintains records of tests performed on BSCs for a minimum of five years. Tests are conducted in accordance with the most recent edition NSF International's standard No. 49, Class II (Laminar Flow) Biohazard Cabinetry.

2. Types of Biological Safety Cabinets (Appendix C)

   a. **Class I BSC**
   The Class I BSC provides personnel and environmental protection but no product protection. It is similar in function to a chemical fume hood but has a HEPA filter in the exhaust system to protect the environment. The Class I BSC is not commonly used on campus.

   b. **Class II BSC (Types A, B1, B2 and B3)**
   Class II cabinets are designed for work involving microorganisms assigned to Biosafety Levels 1, 2 and 3. These cabinets provide the microbe-free work environment necessary for cell culture propagation and may be used for nonvolatile chemotherapeutic drug preparation.

   c. **Class III BSC**
   The Class biological safety cabinet is designed for work with biosafety level IV microbiological agents and provides maximum protection to the operator and the environment.

   d. **Horizontal and Vertical Laminar Flow "Clean Bench"**
   Horizontal and vertical laminar-flow clean-air benches are not BSCs. They discharge HEPA-filtered air across the work surface and toward the user. These devices provide only product protection.

3. Procedures for Use of BSCs

a. Start-up Procedure
    1. Turn off ultraviolet sterilizer if so equipped.
    2. Turn on all blowers and cabinet illumination lights.
    3. Allow five minutes of operation to purge system and check flow alarm system audio and visual alarm function if so equipped.
    4. Decontaminate readily accessible interior surfaces with a disinfectant appropriate for the agents or suspected agents present.

b. Working in the BSC
    1. Checking the magnehelic gauge regularly for an indication of a problem.
    2. Do not disrupt the protective airflow pattern of the BSC. Such things as rapidly moving your arms in and out of the cabinet, people walking rapidly behind you and open lab doors may disrupt the airflow pattern and reduce the effectiveness of the BSC.
    3. Plan you work prior to starting.
    4. Minimize the storage of materials in and around the BSC.
    5. Always leave the BSC running.

c. Operational Directions
    1. Before using, wipe work surface with 70% alcohol or other appropriate disinfectant. Wipe off each item you need for your procedures and place them in cabinet.
    2. **Do not** place objects over the front air intake grille. **Do not** block the rear exhaust grille.
    3. Segregate contaminated and clean items. Work from “clean to dirty.”
    4. Place a pan with disinfectant and/or a sharps container inside the BSC for pipette discard. **Do not** use vertical pipette discard canisters on the floor outside cabinet.
    5. It is not necessary to flame items. This creates turbulence in airflow and will compromise sterility; heat buildup may damage the filters.
    6. Move arms slowly when removing or introducing new items into the BSC.
    7. If you use a piece of equipment that creates air turbulence in the BSC (such as a centrifuge or blender), place equipment in the back one-third of the cabinet; stop other work while equipment is operating.
    8. Protect the building vacuum system from biohazards by placing a cartridge filter between the vacuum trap and the source valve in the cabinet.
    9. Clean up all spills in the cabinet immediately. Wait 10 minutes before resuming work.
    10. When work is finished, remove all materials and wipe all interior surfaces with 70% alcohol or other appropriate disinfectants.

11. Remove lab coats and wash hands thoroughly before leaving the laboratory.

d. Shutdown Procedures
    1. Decontaminate and remove all items from the interior work area.
    2. Decontaminate readily accessible interior surfaces with a disinfectant appropriate for the agents or suspected agents present.
    3. Turn on the ultraviolet sterilizer if so equipped.
    4. Allow five minutes of operation to purge the system.
    5. Turn off the cabinet blower.

B. Waste Management

1. Medical/Biohazardous Waste

   All waste defined as medical waste by the Medical Waste Management Act (MWMA) must be properly treated before disposal or before being collected, treated and disposed of by an approved medical waste company. Most waste on campus is treated through the use of an approved medical waste company. If waste generated in a facility meets the definition of medical waste, it must be properly treated before disposal.

2. Medical waste is defined as biohazardous waste and sharps waste.
    a. Biohazardous wastes include:
        1. All liquid and solid waste generated while collecting, producing, processing, testing, immunizing, treating and/or storing specimens from humans or animals (vertebrate, invertebrate, wild or laboratory) that are known or reasonably suspected of containing agents infectious to humans. Biohazardous wastes also include cultures of infectious agents (i.e., bacteria, fungi, rickettsia, helminths, insects, prions, protozoa, and viruses) classified as Biosafety Level 2 or greater with evidence of human pathogenicity (Biosafety in Microbiological and Biomedical Laboratories, U.S. Public Health Service – CDC/NIH).

        2. All human anatomical remains (except teeth) and any fluid human blood and blood products.

        3. Excluded from the MWMA are items such as microbiological cultures used in food processing and biotechnology, except for genetically altered organisms or recombinant nucleic acid or synthetic nucleic acid molecules that are medical waste by definition or that are infectious to humans.

    b. Sharps waste includes any device having acute rigid corners, edges or protuberances capable of cutting or piercing, including:

1. Hypodermic needles, hypodermic needles with syringes, blades, needles with attached tubing, syringes contaminated with biohazardous waste, acupuncture needles and root canal files.

2. Broken glass items, such as Pasteur pipettes and blood vials contaminated with biohazardous waste.

3. Rigid plastics that could pierce a plastic bag such as serological pipets or pipette tips.

3. The main provisions of the MWMA for UC Merced include the following:
   a. Generators must register their facility as a medical waste generator and develop a specific waste management plan for their facility.

   b. Medical Waste must be contained separately from other waste in the lab. Medical sharps waste must be contained in approved medical waste sharps container. Prior to first use, sharps containers must be labeled with "University of California, Merced", address of facility, PI's name, and the building and room number were waste was generated.

   c. Medical waste must be placed in **RED** biohazard bags labeled with the words "Biohazardous Waste" or with the biohazard symbol and the word "Biohazard", and must be certified to meet the ASTM D1709-01 dart test requirements. Liner bags (the outer bag) must be certified to meet the ASTM D1709-01 165 gr dart test and the 480gr tear strength ASTM D1922-00A standards. In addition the bag must be labeled with "University of California, Merced", address of facility, PI's name, and the building and room number were waste was generated. Bags should be labeled before adding waste. Non-biohazardous waste should not be placed in labeled red biohazard bags.

   d. Medical waste and sharps waste cannot be stored for more than seven days unless approved by EH&S.

   e. Medical waste bags must be stored, handled and transported in properly labeled, leak proof secondary containers with tight-fitting covers.

   f. Bagged medical waste that has been treated according to MWMA requirements is considered solid waste and may be disposed through the routine campus refuse collection and disposal system. Alternatively, properly bagged and labeled solid biohazardous waste should be placed in the red bins provided by EH&S in locations set up and maintained by EH&S.

4. Sterilizing Medical Waste

   Currently, the University of California, Merced does not have any

autoclaves permitted for the sterilization of medical waste.
Permits must be obtained 90 days before treating medical waste by EH&S. Once a permit is obtained, the following procedures must be followed:

a. Solid medical/biohazardous waste must be autoclaved at 121 degrees Centigrade (250 degrees Fahrenheit) for a minimum of 60 minutes on a liquid cycle.

b. **Red** autoclave bags and sharps containers must be placed in a secondary container and loaded into the autoclave. Polypropylene, polycarbonate, or stainless steel tubs are typically used. Please note that polyethylene containers may not be used.

c. Biohazard bags and sharps containers must be labeled with "University of California, Merced", address of facility, PI's name, and the building and room number were waste was generated. Autoclaves must not be loaded beyond approved capacity.

d. Autoclave indicator tape should be placed on the outside of each bag prior to treatment. Color change of this tape should be verified after sterilization.

e. The autoclave log must be completed by each user for each autoclave cycle. All log parameters must be filled out completely.

f. The autoclave must be operating with the history tape working. EH&S monitors the history of the autoclave to ensure it is working properly.

g. If the autoclave does not attain the minimum time and/or temperature, EH&S must be notified. If minimum time and temperature is not attained on the second cycle, users must contact the person responsible for maintaining the unit to initiate repairs. Waste should then be sent off-site for proper disposal.

h. After autoclave bags have sufficiently cooled to handle, dispose of them in a solid-waste container.

i. Thermometers on the autoclave must be calibrated annually, and a written record must be maintained. This should be done by an authorized autoclave service company during routine servicing. Please forward all calibration and service records to EH&S.

j. Monthly *Bacillus stearothermophilus* tests must be performed in conjunction with a biological control. Written results of tests and controls are maintained by EH&S.

k. All records, including calibration results and *Bacillus stearothermophilus* tests must be kept for a minimum of three years.

l. Biohazardous liquid waste should be sterilized by diluting with liquid bleach. Use one part bleach to nine parts liquid waste. Wait 20 minutes, than pour down drain. Occasionally liquid waste is autoclaved. Do not add bleach in this case.

m. Sharps waste handling and disposal is as follows:
   1) Sharps must not be placed directly in red bags. Sharps must be placed into rigid, puncture-and leak-resistant sharps containers that cannot be opened without great difficulty. Needles and syringes should be placed directly in these containers after use without modifications. Needles should not be clipped, bent, recapped or removed from disposable syringes before disposal. In addition, do not fill above the level indicated on the container.

   2) Place autoclave indicator tape over biohazard symbol.

   3) Sharps containers should be autoclaved as medical waste.

C. Emergency Procedures

1. Accidents
   All biohazard laboratories must complete a Laboratory Safety Plan Supplement (LSPS). The laboratory specific LSPS should provide emergency response information to lab members. The emergency response information must take into consideration the use of radioactive materials and chemicals, if applicable. The following items should be noted for the type of biohazardous agent used in the laboratory:

   a. First, attend to any injured personnel. Call 9-911 from a campus landline or 911 from a cell phone for emergency assistance, and inform responders of biohazards that may be a threat.

   b. For spills in BL-2 laboratories, evacuate the room and close the doors.

   c. Wait 30 minutes before reentering to allow droplets and aerosols to settle.

   d. After evacuating the area, wait to assist emergency responders.

   e. Notify EH&S during work hours at 228-4639 or 228-2347 about a spill outside of containment of a biohazardous agent.

2. Exposures
   a. Clean exposed skin/needle stick area with soap and water for 15 minutes. Do not use soap if it is an eye exposure.
   b. Report exposure to supervisor, and seek medical treatment as needed.

   c. Report the exposure to the EH&S biosafety officer at 228-4639 or 228-2347 for a review of laboratory protocols and procedures.

3. Biohazard Spill
   The following procedures are provided as a guideline to biohazardous spill cleanup. Appropriate lab coats, gloves and other PPE should be worn in the laboratory at all times.

   a. Inside the Biosafety Cabinet (BSC)
      1) Apply appropriate disinfectant and allow a minimum of 20 minutes contact time while allowing the cabinet to run.

      2) Wipe up spillage with disposable, disinfectant-soaked paper towels.

      3) Wipe the walls, work surface and any equipment in the cabinet with disinfectant-soaked paper towels.

      4) Discard contaminated disposable materials in appropriate biohazardous waste container(s) and autoclave before discarding as biohazardous waste.

      5) Place contaminated reusable items in separate biohazard bags and autoclavable pans with lids before autoclaving and cleanup.

      6) Expose non-autoclavable materials to disinfectant for a minimum of 20 minutes before removing them from the BSC.

      7) Remove protective clothing used during cleanup and place in a biohazard bag for autoclaving.

      8) Run the cabinet for a minimum of 10 minutes after cleanup before resuming work or turning off the cabinet.

   b. In the lab and outside the BSC
      1) If the organisms are transmitted through aerosols, WITHOUT FIRST INHALING, HOLD YOUR BREATH AND LEAVE THE ROOM IMMEDIATELY. Wait at least 30 minutes for droplets and aerosols to settle before reentering spill area.

      2) Remove any contaminated clothing and place in biohazard bag to be autoclaved.
      3) Wear a disposable gown, safety glasses and gloves.

      4) Initiate cleanup with disinfectant as follows:
         a. Soak paper towels in disinfectant and place over the spill.
         b. Encircle the spill with additional disinfectant, being careful to minimize aerosolization while assuring adequate contact.
         c. Decontaminate all items within the spill area.
         d. Allow a minimum of 20 minutes contact time to ensure germicidal action of disinfectant, and wipe up the spill with more paper towels.
         e. Clean the spill area with fresh towels and disinfectant.

f. Place disposable contaminated spill materials in red biohazardous waste bags for autoclaving.
g. Place contaminated reusable items in biohazard bags or autoclavable pans with lids before autoclaving and cleanup.

c. Outside lab, during transport
   1) Transport biohazardous material in an unbreakable, well-sealed primary container placed inside of a second unbreakable lidded container labeled with the biohazard symbol. The container can be a cooler, plastic pan or pail.

   2) Should a spill occur in a public area, do not attempt to clean it up without appropriate PPE.

   3) As an interim measure, wear gloves and place paper towels, preferably soaked in disinfectant, directly on spilled materials to prevent spread of contamination. To assure adequate contact, surround the spill with disinfectant, if available, taking care to minimize aerosols. Notify EH&S immediately.

D. Chemical Disinfectants

1. Chemical disinfectants are used to decontaminate surfaces used for biological experiments. Some of the factors influencing selection of a disinfectant are:
   a. The nature of the biological agent.
   b. The type of surface to be disinfected.
   c. The amount of contact time required to inactivate the biological agent when using the selected disinfectant.
   d. The volume of disinfectant that will be required to inactivate the biological agent.
   e. The toxicity of the chemical disinfectant.
   f. Whether the disinfectant will chemically react with the samples to be disinfected.

2. When using a chemical disinfectant, remember that you are using a potentially toxic chemical that could be a corrosive, flammable solvent and/or a carcinogen. Wear PPE as indicated on the product container and Safety Data Sheets (SDS). If you must prepare a dilution of the disinfectant, do so whenever possible in a chemical fume hood or in a well-ventilated area. If you are working with mixed solutions, check the MSDS to insure that any incompatible chemical reaction will not result.

3. Allow sufficient contact time after applying the disinfectant. Do not apply a disinfectant and immediately remove it from the contaminated surface, as the contact time will be too brief to ensure that the surface has been thoroughly disinfected. When cleaning a spill of concentrated material or if the disinfectant must act on an uneven surface, allow extra time for the disinfectant to act.

4. Avoid using concentrated or undiluted solutions of your disinfectant to speed up the inactivation process. Undiluted chemicals may adversely affect the surface being cleaned. This is especially significant when working with bleach, which is a very strong

corrosive. Some disinfectants will leave a residue. Rinse the cleaned area with distilled water to avoid adverse affects on the experiment after allowing sufficient contact time. This is especially important in tissue culture rooms where a cell line can be destroyed by disinfectant residue left on equipment.

5. The following are approved disinfectants:
    a. Chlorine compounds
    b. Ethyl alcohol
    c. Quarternary ammonium compounds
    d. Phenolics
    e. Iodophors
    f. Paraformaldehyde
    g. Formaldehyde
    h. Glutaraldehyde

    Disinfectants are Pesticides

    a. The U.S. Environmental Protection Agency (EPA) and California Environmental Protection Agency define antimicrobials such as disinfectants, sanitizers and bacteriostats as pesticides. Worker safety requirements for pesticides are similar to those for other hazardous materials administered by Cal-OSHA.

    b. The lead agencies in California are Cal-OSHA and the Department of Pesticide Regulation (DPR). Local enforcement is under the jurisdiction of the County Agricultural Commissioner (CAC).

    c. Requirements pertaining to material use, labeling, hazard communication, exposure records, training, respiratory protection, safety equipment, lighting and equipment maintenance must be followed. **All requirements on the label must be followed when using the product.**

E. Biohazard Labels and Signs

1. Posting of Rooms
   The California Code of Regulations, Title 8, Section 5193, the CDC/NIH document *Biosafety in Microbiological and Biomedical Laboratories* and NIH's *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* require that signs indicating that biohazardous agents are used within the room be posted at or on access doors. The sign must include the universal biohazard symbol, specific entry requirements, and the name and telephone number of the PI and/or other responsible persons. Areas that require posting are:

   Entrances to laboratories that use biohazards classified as Biosafety Level 2 (BL-2) or Biosafety Level 3 (BL-3); and

a. Bloodborne pathogen regulations and biological agent use require labels to be placed on equipment such as refrigerators, freezers, incubators, shipping containers, primary and secondary agent containers, and any surface that may be reasonably anticipated to have surface contamination from biohazardous agents. The label must include the universal biohazard symbol and the word "Biohazard."

b. All medical waste except sharps must be placed in red bags that are labeled with the international biohazard symbol and the words "Biohazard" or "Biohazardous Waste," "University of California, Merced," address of facility, PI's name, and building and room number were waste was generated.

c. Medical waste sharps containers must also be labeled as above with the biohazard symbol and wording as well as the location information.

d. Secondary containers for medical waste must be labeled on two sides and on the lid with the biohazard symbol and wording.

F. Transporting and Shipping

1. Transportation outside of the laboratory
   a. Biohazardous agents must be properly handled, contained and labeled to transport between locations to prevent accidental exposure to unsuspecting persons outside of the laboratory.

   b. Biohazardous agents must be placed in securely closed primary containers. The exterior of the primary container should be decontaminated prior to transportation.

   c. The primary container should be placed in a covered, leak proof, shatterproof secondary container. The secondary container should be labeled with the biohazard symbol, the biohazardous agents present and the lab of origin. If it is transported by vehicle, the name and telephone number of the PI or other responsible person(s) must be included on the outside of the secondary container.

2. Shipment off campus (domestic shipment)
   a. Three federal regulatory agencies specify requirements for packaging and shipping of biological materials. The United Nations publishes recommendations for packing and shipping biological materials, and both the International Civil Aeronautics Organization and International Air Transport Association (IATA) publish regulations based on the UN's recommendations. The requirements for all these regulations are similar; therefore, most carriers elect to follow the IATA regulations set forth in their Dangerous Goods Regulations (DGR). Information regarding requirements, lists and authorized shipping labels can be obtained from EH&S.

   b. When transporting infectious agents, the shipper is responsible for the proper packing of dangerous goods and must pack biological agents as infectious substances (Packing Instruction 602, IATA-DGR) or diagnostic specimens

(Packing Instruction 650, IATA-DGR). The following are packing instructions for infectious substances:

i. Primary Container
   a. The specimen will be placed in a securely closed, watertight primary container. Stoppers and screw-capped tubes will be secured with waterproof tape.
   b. The contents of the primary containers will not exceed 50 ml.
   c. The exterior of the primary container will be decontaminated prior to transportation.
   d. A biohazard label will be placed on the exterior of the primary container (see Figure 1).

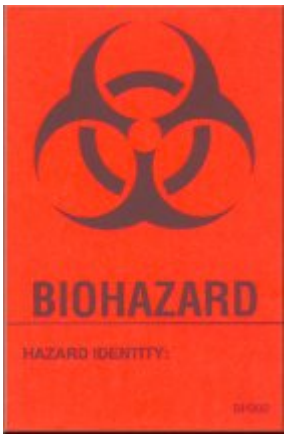

Figure 1. Biohazard Label

ii. Secondary Container
   a. One (or more) primary container(s) may be placed within the secondary container as long as the total volume of the specimen does not exceed 50 ml.
   b. The absorbent material used within the secondary container must be sufficient to absorb the contents of the primary container(s), if it should leak.
   c. The secondary container must be free of contamination and labeled with the same symbol as the primary container.

iii. Outer Container
   a. This container will be made of corrugated fiberboard, cardboard, wood or other material of equivalent strength.

   b. The interior of the outer container may be filled with coolant material such as ice or dry ice. If ice or dry ice is used, additional shock absorbent material will be added and positioned in a manner that allows protection of the specimen should the ice or dry ice melt or sublimate. The dry ice should be placed outside of the secondary container in the outer container. An additional placard is required with the mass of dry ice indicated on the placard.

c. The exterior will be labeled with the special sticker depicted in Figure 2.

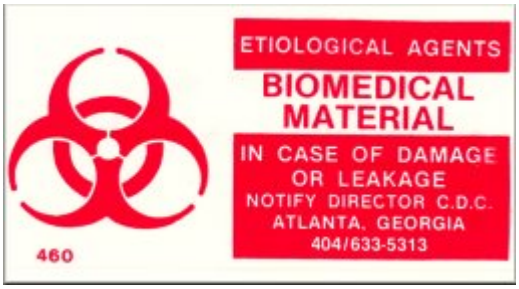

Figure 2. Etiologic Agents

d. Prior to transport, the outer container should be sealed or secured in a manner as to make it leak-proof should the container be placed on its side.

e. The package will be decontaminated before shipment.

f. The package shall be sent by UC Merced shipping and receiving.

3. International Shipments
   All domestic and international shipments of infectious substances require the use of packaging that has been tested and certified to carry such material.

   a. The certified packaging will have United Nations performance markings on the outside indicating that it has met performance tests.

   b. A statement should be included in the additional handling information that states, “Prior arrangements as required by the IATA Dangerous Goods Regulations 1.3.3.1 have been made.”

   c. The shipper should include the name and telephone number of the person responsible for the shipment.
   d. Diagnostic specimens being shipped for the purpose of initial diagnosis are excluded from the regulations. However, diagnostic specimens known, or thought likely, to contain infectious substances are included.

   e. All shipments will go through UC Merced Shipping and Receiving.

4. Receipt
   a. Upon receipt of any packaged specimens, immediately check for leakage or damage.

   b. If leaking:
      1) Isolate the package either in a Class II biological safety cabinet or in a leak-proof, sealed container. Add disinfectant and dispose of as medical waste.

2) Call EH&S at 228-4639 or 228-2347 if BL-3 agents are involved. Submerge contents in 10% bleach.

3) Keep unauthorized personnel away from the package.

c. The package should be opened in the laboratory on an easily cleaned, water-resistant surface.

Appendix A

Biosafety Levels for Infectious Agents and Infected Animals

Bacterial Agents	BSL	Comments
Actinetobacter calceticus	2	
Actinobacillus sp.	2	
Actinomyces sp.	2	
Aeromaonas sp.	2	
Arachnida propionica	2	
Bacillus alvei	2	
Bacillus anthracis*	2	BMBL, vaccination recommended
Bacteroides sp.	2	
Bartonella sp.	3	
Bordetella sp.	2	
Bordetella pertussis	2	BMBL
Borrelia sp.	2	
Brucella sp.*	2/3	BMBL
Campylobacter fetus var. jejuni	2	BMBL
Camplobacter sp.	2	
Chlamydia psittaci	2	BMBL
Chlamydia pneumoniae	2/3	BMBL
Chlamydia trachomatis	3	
Clostridium botulinum*	2/3	BMBL
Clostridium tetani	2	BMBL
Corynebacterium diphtheriae	2	BMBL
Corynebacterium equi	2	
Corynebacterium haemolyticum	2	
Corynebacterium pseudotuberculosis	2	
Corynebacterium pysogenes	2	
Corynebacterium renale	2	
Enterobacteriaceae all other	2	
Erysipelothrix rhusiopathiae	2	
Escherichia coli	2	
Escherichia coli K12 derivative	1	
Francisella tularensis*	2/3	BMBL
Fusobacterium sp.	2	
Haemophilus sp.	2	
Klebsiella sp.	2	
Legionella pneumophilia	2/3	BMBL
Leptospira interrogans all servars	2	BMBL
Listeria sp.	2	
Moraxella sp.	2	
Mycobacterium avium	2	

Bacterial Agents	BSL	Comments
Mycobacterium bovis	3	BMBL
Mycobacterium leprae	2	BMBL
Mycobacterium sp.	2	BMBL
Mycobacterium tuberculosis	2/3	BMBL
Mycoplasma sp.	2	
Neisseria gonorrhoreae	2/3	BMBL
Neisseria menegitidis	2/3	BMBL
Nocardia sp.	2	
Pasteurella sp.	2	
Pseudomonas aeruginosa	2	NIH
Pseudomonas mallei	2/3	BMBL
Neisseria gonorrhoeae	2/3	BMBL
Pseudomonas testoserone	2	
Rotococcus (Coryne.) equi	2	
Salmonella sp.	2	BMBL
Salmonella typhi	2/3	BMBL
Shigella sp.	2	BMBL
Staphylococcus sp.	2	
Streptococcus sp.	2	
Streptocacillus moniliformis	2	
Streptomyces somaliensis	2	
Treponema pallidum	2	BMBL
Vibrio sp.	2	BMBL
Yersinia pestis*	2/3	BMBL, immunization recommended

BMBL - Agent summary and biosafety levels according to type of activities are listed in the CDC/NIH's Biosafety in Microbiological and Biomedical Laboratories 5th Edition.
V - Vaccination is recommended for personnel.
***** - Select agents

Fungal Agents	BSL	Comments
Blastomyces dermatitides	2	BMBL
Coccidioides immitis*	2/3	BMBL
Cryptococcus neoformans	2	BMBL
Epidermophyton - pathogenic sp.	2	BMBL
Histoplasma capsulatum	2/3	BMBL
Microsporum - pathogenic sp.	2	BMBL
Paracoccidioides brasilienisis	2	
Sporothrix schenckii	2	BMBL
Trichophyton - pathogenic sp.	2	BMBL
Candida albicans	2	
Miscellaneous Molds	2	BMBL

BMBL - Agent summary and biosafety levels according to type of activities are listed in the CDC/NIH's Biosafety in Microbiological and Biomedical Laboratories 5th Edition.
V - Vaccination is recommended for personnel.
***** - Select agents

Parasitic Agents	BSL	Comments
Anaplasma sp.	2	
Ascaris sp.	2	BMBL
Coccidia sp.	2	BMBL
Cryptosporidia sp.	2	BMBL
Echinococcus Granulosus	2	BMBL
Ehrlichia sp.	2	
Entamoeba sp.	2	BMBL
Enterobius sp.	2	BMBL
Fasciola sp.	2	BMBL
Giardia sp.	2	BMBL
Haemobartonella sp.	2	
Hymenolepsis nana	2	BMBL
Leishmania sp.	2	BMBL
Leukocytozoon sp.	2	
Naegleria sp.	2	
Plasmodium sp.	2	BMBL
Sarcocystis sp.	2	BMBL
Schistosoma sp.	2	BMBL
Strongyloides sp.	2	BMBL
Taenia solium	2	
Toxocara canis	2	
Toxoplasma sp.	2	BMBL
Trichinella spiralis	2	BMBL
Trypanosoma sp.	2	BMBL

BMBL - Agent summary and biosafety levels according to type of activities are listed in the CDC/NIH's Biosafety in Microbiological and Biomedical Laboratories 5th Edition.
V - Vaccination is recommended for personnel.
***** - Select agents

Rickettsial Agents	BSL	Comments
Coxiella burnetii*	2/3	BMBL
Rickettsia akari	2/3	
Rickettsia australis	2/3	BMBL
Rickettsia canada	2/3	BMBL
Rickettsia conorii	2/3	BMBL
Rickettsia prowazekii*	2/3	BMBL
Rickettsia rickettsii*	2/3	BMBL
Rickettsia siberica	2/3	BMBL
Rickettsia tsutsugamushi	2/3	BMBL

Rickettsial Agents	BSL	Comments
Rickettsia typhi (R. mooseri)	2/3	BMBL
Rochalimaea quintana	2	
Rochalimaea vinsonii	2	
Spotted Fever Group - other	2/3	

BMBL - Agent summary and biosafety levels according to type of activities are listed in the CDC/NIH's Biosafety in Microbiological and Biomedical Laboratories 5th Edition.
V - Vaccination is recommended for personnel.
***** - Select agents

Viral Agents	BSL	Comments
Adenoviruses	2	
Adenoviruses - animal - all	2	
Aleutian Disease Virus	2	
Arboviruses - certain	2	BMBL
Arboviruses - certain	3	BMBL
Arboviruses - certain	4	BMBL
Arenaviruses - certain	3	BMBL
Arenaviruses - certain	4	BMBL
Avian Erthyroblastosis Virus	2	
Avian Leucosis Virus	2	
Avian Lymphomatosis Virus	2	
Avian Myeloblasotosos Virus	2	
Bovine Encephalomyelitis Virus	2	
Bovine Leukemia Virus	2	
Bovine Respiratory Syncytial Virus	2	
Bovine Rhinotracheitis (IBR)	2	
Cache Valley Virus	2	BMBL
Canine Hepatitis Virus	2	
Canine Distemper Virus	2	
Caprine Arthritis	2	
Coxsackie A & B Viruses	2	
Cytomegaloviruses	2	
Encephalomyelitis Virus*	2	
Echovirus	2	
Dengue Virus	2	BMBL
Encephalomyocarditis Virus	2	
Epidemic Diarrhea Infant Mice	2	
Epstein-Barr Virus	2	
Feline Leukemia Virus	2	
Feline Sarcoma Virus	2	
Filoviruses	2	
Flanders Virus	2	BMBL
Gibbon Ape Lymphosarcoma	2	
Hart Park Virus	2	BMBL

Viral Agents	BSL	Comments
Hemorrhagic Fever Agents*	2	
Hepatitis A Virus, Hepatitis E Virus	2	BMBL
Hepatitis B Virus, Hepatitis C Virus, Hepatitis D Virus	2	BMBL
Herpesvirus - other	2	
Herpesvirus ateles	2	
Herpesvirus saimir	2	
Herpesvirus Simiae (B-virus)	3	BSL-2, -3 or -4 depending on activity, BMBL
Human Herpesviruses	2	BMBL
Hog Cholera Virus	2	
Human T-Cell Leukemia Virus I & II	2	
Infectious Bronchitis Virus	2	
Influenza Virus	2	BMBL
Influenza Virus Virulent Avian	3	
K (Rate) Virus	2	
Lactic Dehydrogenase Elevating	2	
Langat Virus	2	BMBL
Laryngotracheitis Virus	2	
Lassa Virus*	4	BMBL
Low Risk Oncogenic Viruses	2	
Lymphocytic Choriomeningitis Virus	2/3	BMBL
Marburg Virus*	4	BMBL
MERS-CoV	3	NIH
Measles Virus	2	
Memingopneumonitis Virus	2	
Mouse Encephalomyelitis Virus	2	
Mouse Hepatitis Virus	2	
Mouse Leukemia Virus	2	
Mouse Pneumonia Virus	2	
Mumps Virus	2	
Myxomatosis Virus	2	
Newcastle Disease Virus	2	
Newcastle Disease Virus (VVND)	2	
Non-Defective Adenovirus 2SV40 HYB	2	
Papilloma Virus Shope	2	
Parainfluenza Virus	2	
Poliovirus - all types	2	BMBL
Polyoma Virus	2	
Poxvirus alastrim	2	
Poxvirus monkey pox	3	
Poxvirus - Smallpox*		restricted use by WHO
Poxvirus sp.	2	BMBL
Pseudorabies Virus	2	
Rabies Virus	2/3	BMBL

Viral Agents	BSL	Comments
Reovirus sp.	2	
Respiratory Syncytial Virus	2	
Retroviruses, including HIV & SIV	2/3	BMBL
Rhinovirus sp.	2	
Rous Sarcoma Virus	2	
Rubella Virus	2	
Simian Virus - other	2	
Simian T-Cell Leukemia Virus	2	
Sindbis Virus	2	
Slow Viruses	2	
Tensaw Virus	2	
Tick-Borne Encephalitis Complex	4	
Transmissible Spongiform Encephalopathies (Creutzfeldt-Jakob, kuru, and related agents	2	BMBL
Turlock Virus	2	
Vaccinia Virus	2	
Venezuelan Equine Encephalitis*	3	
Vesicular Stomatitis - lab adapted	2	BMBL
Vesicular Somatitis Virus	3	BMBL
Woolly Monkey Fibrosarcoma	3	
Yaba Virus	2	
Yellow Fever Virus 17D Strain*	2	BMBL
Yellow Fever Virus Except 17D*	3	BMBL

BMBL - Agent summary and biosafety levels according to type of activities are listed in the CDC/NIH's Biosafety in Microbiological and Biomedical Laboratories 5th Edition.
V - Vaccination is recommended for personnel.
***** - Select agents

Appendix B

Material Regulated as Select Agents & Toxins

The United States Department of Health and Human Services (HHS) and the United States Department of Agriculture (USDA) have identified bacteria, viruses, toxins, rickettsia, and fungi that pose a potential threat to public health or welfare. These organisms are considered Select Agents and High Consequence Livestock Pathogens and Toxins.

Materials regulated as Select Agents are listed below. If you use, or intend to use, any of these agents, contact the EH&S office at (209) 228-7864 or (209)228-4639. Some exemptions and prohibitions apply.

Select Agents & Toxins	
Viruses	**Toxins**
African horse sickness virus [1]	Abrin [3]
African swine fever virus [1]	Botulinum neurotoxins [2]
Akabane virus [1]	*Clostridium perfringens* epsilon toxin [2]
Avian influenza virus (highly pathogenic) [1]	Conotoxins [3]
Blue tongue virus (exotic) [1]	Diacetoxyscirpenol [3]
Camel pox virus [1]	Ricin [3]
Cercopithecine herpes virus (Herpes B virus) [3]	Saxitoxin [3]
Classical swine fever virus [1]	Shigatoxin and Shiga-like ribosome inactivating proteins [2]
Crimean-Congo haemorrhagic fever virus [3]	Staphylococcal enterotoxins [2]
Eastern equine encephalitis virus [2]	Tetrodotoxin[3]
Ebola viruses [3]	T–2 toxin [2]
Foot and mouth disease virus [1]	
Goat pox virus[1]	Bacteria
Japanese encephalitis virus [1]	*Bacillus anthracis* [2]
Lassa fever virus [3]	Botulinum neurotoxin producing strains of *Clostridium* [2]
Lumpy skin disease virus [1]	*Brucella abortus* [2]
Malignant catarrhal fever [1]	*Brucella melitensis* [2]
Marburg virus [3]	*Brucella suis* [2]
Menangle virus [1]	*Burkholderia mallei* [2]
Monkey pox virus [1]	*Burkholderia pseudomallei* [2]
Newcastle disease virus (exotic) [1]	*Coxiella burnetii* [2]
Nipah and Hendra complex viruses [2]	*Cowdria ruminantiun* (Heartwater) [1]
Peste des petits ruminants [1]	*Francisella tularensis* [2]
Plum pox potyvirus[4]	*Liberobacter africianus, Liberobacter asiaticus* [4]
Rift Valley fever virus [2]	*Mycoplasma capricolum* / *M.* F38 / *M. mycoides capri* (contagious caprine pleuropneumonia agent) [1]
Rinderpest virus [1]	*Mycoplasma mycoides mycoides* (contagious bovine pleuropneumonia agent) [1]
Sheep pox [1]	*Ralstonia solanaceanon Race 3* [4]
South American haemorrhagic fever viruses (Junin, Machupo, Sabia, Flexal, Guanarito) [3]	*Rickettsia prowazekii* [3]
Swine vesicular disease virus [1]	*Rickettsia rickettsii* [3]
Tick-borne encephalitis complex (flavi) viruses (Central European Tick-borne encephalitis, Far Eastern Tick-borne encephalitis, Russian Spring and Summer encephalitis, Kyasanur Forest disease, Omsk Hemorrhagic Fever) [3]	*Xanthomonas oryzae pv. oryzicola* [4]

Viruses	Toxins
Variola major virus (Smallpox virus) and Variola minor (Alastrim) [3]	*Xylella fastidiosa* (citrus variegated chlorosis strain) [4]
Venezuelan equine encephalitis virus [2]	*Yersinia pestis* [3]
Vesicular stomatitis virus (exotic) [1]	
Prions	**Fungi**
Bovine spongiform encephalopathy agent [1]	*Coccidioides immitis* [2]
	Coccidioides posadasii [3]
	Peronoscleospora philippinensis [4]
	Phakopsora pachyrhizi [4]
	Sclerophthora rayssiae var zeae [4]
	Synchytriun endobioticum [4]
[1]USDA High Consequence Livestock Pathogens or Toxin	
[2]USDA/HSS Overlap Agent	
[3]HHS Select Infectious Agent	
[4]APHIS Plant Pathogens (Animal and Plant Health Inspection Service, a division of USDA)	

Genetic elements, recombinant nucleic acids, and recombinant organisms listed below are regulated as Select Agents:

1. Select Agent viral nucleic acids (synthetic or naturally derived, contiguous or fragmented, in host chromosomes or in expression vectors) that can encode infectious and/or replication competent forms of any of the Select Agent viruses.

2. Nucleic acids (synthetic or naturally derived) that encode for the functional form(s) of any of the listed toxins if the nucleic acids: a) are in a vector or host chromosome, b) can be expressed in vivo or in vitro; or c) are in a vector or host chromosome and can be expressed in vivo or in vitro.

3. Listed viruses, bacteria, fungi, and toxins that have been genetically modified.

Exemptions:

1. **Medical use** of toxins for patient treatment is exempt.

2. **If the aggregate amounts** of the following agents or toxins under the control of a principal investigator do not, at any time, exceed the limits below, they are exempt:

    - 0.5 milligrams (mg) of Botulinum neurotoxins
    - 5 mg of Staphylococcal enterotoxins
    - 100 mg of abrin, *Clostridium perfringens* epsilon toxin, conotoxin, ricin, saxitoxin, shigatoxin, shiga-like ribosome inactivating protein, and tetrodotoxin
    - 1,000 mg of diacetoxyscirpenol and T-2 toxin

3. **Other exempted agents and toxins** are:

    - Any agent or toxin that is in its naturally occurring environment provided it has not been intentionally introduced, cultivated, collected, or otherwise extracted from its natural source.
    - Non-viable Select Agent organisms or nonfunctional toxins.
    - The vaccine stains of Junin virus (Candid #1), Rift Valley fever virus (MP-12), Venezuelan Equine encephalitis virus vaccine strain TC-83.

Prohibited experiments include:

1. Experiments utilizing recombinant DNA that involve the deliberate transfer of a drug-resistance trait to the listed agents 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.

2. Experiments involving the deliberate formation of recombinant DNA containing genes for the biosynthesis of listed toxins lethal for vertebrates at an LD50 < 100 ng/kg body weight.

Note: Contact the UC Merced Biosafety Officer for further information at (209) 228-4639.

Appendix C
Biological Safety Cabinets

EH&S should be consulted for selection, purchase, installation and use of BSCs on campus.

Testing and Certification of BSCs

BSCs must be tested and certified annually or after installation, alterations, maintenance, or earthquake. Testing and certification of BSCs will be performed by an outside contract; contact EH&S for more information (228-4639 or 228-7864). EH&S maintains records of tests performed on BSCs for a minimum of five years. Tests are conducted in accordance with the most recent edition NSF International's standard No. 49, Class II (Laminar Flow) Biohazard Cabinetry.

Types of Biological Safety Cabinets

The similarities and differences in protection offered by the various classes of biosafety cabinets are reflected in Table 3. Use this table in selection and risk assessment of BSCs.

Class I BSC

The Class I BSC provides personnel and environmental protection but no product protection. It is similar in air movement to a chemical fume hood but has a HEPA filter in the exhaust system to protect the environment. In the Class I BSC, unfiltered room air is drawn across the work surface. Personnel protection is provided by this inward airflow. Class I BSCs can be used specifically to enclose equipment (e.g., centrifuges and harvesting equipment) or procedures (e.g. cage dumping or homogenizing tissues) with a potential to generate aerosols. The Class I BSC is HEPA filtered and hard-ducted to the building exhaust system, and the building exhaust fan provides the negative pressure necessary to draw room air into the cabinet.

Class II BSC

The Class II (Types A, Bl, B2, and B3) BSCs provide personnel, environmental and product protection. Airflow is drawn into the front grille of the cabinet, which provides personnel protection. In addition, downward laminar flow of HEPA-filtered air provides product protection by minimizing the chance of cross-contamination along the work surface of the cabinet. Because cabinet air has passed through the exhaust HEPA filter, it is contaminant-free (an environmental protection) and may be recirculated back into the laboratory (Type A) or ducted out of the building (Type B).

HEPA filters are effective at trapping particulates and infectious agents but not at capturing volatile chemicals or gases. Only BSCs that are ducted to the outside should be used when working with volatile toxic chemicals.

All Class II cabinets are designed for work involving microorganisms assigned to Biosafety Levels 1, 2 and 3. Class II cabinets provide the microbe-free work environment necessary for cell culture propagation and also may be used for the formulation of nonvolatile chemotherapeutic drugs.

Class II, Type A

An internal blower draws sufficient room air through the front grille to maintain a minimum average inflow velocity of at least 75 lfpm at the face opening of the cabinet. The supply air flows through a HEPA filter and provides particulate-free air to the work surface. Laminar airflow reduces turbulence in the work zone and minimizes the potential for cross-contamination. The

downward moving air "splits" as it approaches the work surface; part of the air is drawn to the front grille and the remainder to the rear grille. This split generally occurs about halfway between the front and rear grilles and two to six inches above the work surface.

The air is then discharged through the rear plenum into the space between the supply and exhaust filters located at the top of the cabinet. Due to the relative size of these two filters, about 30 percent of the air passes through the exhaust HEPA filter and 70 percent recirculates through the supply HEPA filter back into the work zone. Most Class II, Type A cabinets have dampers to modulate this 30/70 division of airflow.

An unducted Class II Type A cabinet cannot be used for work involving volatile or toxic chemicals. The buildup of chemical vapors in the cabinet by recirculated air and in the laboratory from exhaust air can create health and safety hazards.

Type A cabinet exhaust can be ducted out of the building through an indirect "thimble" connection to an exhaust system or through a canopy hood. It must be done in a manner that does not alter the balance of the cabinet exhaust system. The volume of the exhaust must be sufficient to maintain the flow of room air into the space between the thimble unit and the filter housing. The thimble must be removable or be designed to allow for operational testing of the cabinet. The performance of a cabinet with this exhaust configuration is unaffected by fluctuations in the building exhaust system.

Class II, Type B1

Some biomedical research requires the use of small quantities of certain hazardous chemicals, such as carcinogens. The powdered form of these carcinogens should be weighed or manipulated in a chemical fume hood. Carcinogens used in cell culture or microbial systems require both biological and chemical containment.

The Class II, Type B cabinet originated with the National Cancer Institute (NCI)-designed Type 2 (later called Type B) biological safety cabinet, which was designed for manipulations of minute quantities of these hazardous chemicals with in vitro biological systems. The National Sanitation Foundation (NSF) Standard 49 definition of Type Bl cabinets includes this classic NCI design Type B, as well as cabinets without supply HEPA filters located immediately below the work surface and/or those with exhaust/recirculation downflow splits other than 70/30 percent.

Room air is drawn through the face opening of the cabinet at a minimum inflow velocity of 100 lfpm. As with the Type A cabinet, there is a split in the downflowing air stream just above the work surface. In the Type B cabinet, about 70 percent of the downflow air exits through the rear grille, passes through the exhaust HEPA filter and is discharged from the building. The remaining 30 percent of the downflow air is drawn through the front grille. Since the air which flows to the rear grille is discharged into the exhaust system, activities that may generate hazardous chemical vapors or particulates should be conducted towards the rear of the cabinet. Type Bl cabinets must be hard-ducted to their own dedicated exhaust system. 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 should be installed to sound an alarm 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 in the exhaust system as necessary.

Class II, Type B2

This BSC is a total-exhaust cabinet; no air is recirculated within it. This cabinet provides primary biological and chemical containment. The supply blower draws in room air at the top of the cabinet, passing it through a HEPA filter and down into the work area of the cabinet. The cabinet exhaust system draws air through both the rear and front grilles, capturing the supply air plus the additional amount of room air needed to produce a minimum calculated or measured inflow face velocity of 100 lfpm. All air entering this cabinet is exhausted and passes through a HEPA filter (and other air-cleaning devices such as a carbon filter if needed) prior to being discharged to the outside. Exhausting as much as 1200 cubic feet per minute of conditioned room air makes this cabinet expensive to operate. Should the building or cabinet exhaust 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 1980s usually have an interlock system installed by the manufacturer to prevent the supply blower from operating whenever the exhaust flow is insufficient. Presence of such an interlock system should be verified; systems can be retrofitted if necessary. Exhaust air movement should be monitored by a pressure-independent device.

Class II, Type B3

This biological safety cabinet is a ducted Type A cabinet having a minimum inward airflow of 100 lfpm. All positive-pressure, contaminated plenums within the cabinet are surrounded by a negative air pressure plenum. Thus, leakage in a contaminated plenum will be into the cabinet and not into the environment. As in the Type A cabinet, exhaust can be ducted out of the building through an indirect “thimble” connection to an exhaust system or through a canopy hood with the same requirements for airflow and design.

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, or the work surface can be designed to accept a carboy, a centrifuge or other equipment that requires containment. A rigid plate with armholes can be added if needed. Good cabinet design, microbiological aerosol tracer testing of the modification and appropriate certification 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. Note: EH&S will consult with the manufacturer for design, testing and certification of special application BSCs.

Class III BSC

The Class III biological safety cabinet is designed for work with biosafety level 4 microbiological agents and provides maximum protection to the environment and the worker. It is a gas-tight enclosure with a nonopening view window. Access for passage of materials into the cabinet is through a dunk tank or double-door pass-through box (such as an autoclave) that can be decontaminated between uses. Reversing this process allows the safe removal of materials from the Class III biosafety cabinet. Both supply and exhaust air are HEPA filtered. Exhaust air must pass through two HEPA filters before being discharged to the outdoors. Airflow is maintained by a dedicated independent exhaust system exterior to the cabinet, which keeps the cabinet under negative pressure. Long, heavy-duty rubber gloves are attached in a gas-tight manner to ports in the cabinet and allow for manipulation of the materials isolated inside.

Horizontal and Vertical Laminar-flow “Clean Benches”

Horizontal and vertical laminar-flow clean-air benches are not BSCs. They discharge HEPA-filtered air across the work surface and toward the user. These devices provide only product protection. They can be used for certain clean activities, such as the dust-free assembly of sterile equipment or electronic devices. These benches should not be used when handling cell culture materials or drug formulations or when manipulating potentially infectious materials. The worker can be exposed to materials being manipulated on the clean bench, which may cause hypersensitivity. Horizontal and vertical clean air benches should never be used as a substitute for a biological safety cabinet in research, biomedical or veterinary laboratories and/or applications.

Table 3: Comparison of Biological Safety Cabinet Characteristics and Applications

Class Type	Work Opening	Inflow Velocity (fpm)	Percentage of Re-circulated Air	Percentage of Exhausted Air	Approximate Exhaust Volume (cfm)	Exhaust Requirement	Application
Class I	Fixed	75	0%	100%	4 ft-200 6 ft-300	Exhausted to the outside (remote fan) or to the room through a HEPA filter (integral fan)	BL-1 through BL-3; small amounts of toxic chemicals or radionuclides (if exhausted to outside)
Class II, Type B1	Sliding	100	30%	70%	4ft-250 6ft-400	Exhausted to outside with remote fan; duct is hard-connected.	BL-1 through BL-3; small amounts of toxic chemicals or radionuclides
Class II, Type B2	Sliding, hinged	100	0%	100%	4ft-600 6ft-1,000	Exhausted to outside with remote fan; duct is hard-connected.	BL-1 through BL-3; small amounts of toxic chemicals or radionuclides
Class II, Type B3	Sliding, hinged	100	70%	30%	4ft-300 6ft-400	Exhausted to outside with remote fan utilizing thimble or hard-connected duct.	BL-1 through BL-3; small amounts of toxic chemicals or radionuclides
Class III	Glove ports	n/a	0%	100%	__*	Exhausted to outside through two HEPA filters with remote fan; duct is hard-connected.	BL-1 through BL-4; small amounts of toxic chemical or radionuclides
Class II, Type B1	Sliding	100	30%	70%	4ft-250 6ft-400	Exhausted to outside with remote fan; duct is hard-connected.	BL-1 through BL-3; small amounts of toxic chemicals or radionuclides

*Class III cabinets should have about 20 air changes per hour or enough ventilation to accommodate the heat load. A negative pressure of 0.5 in water gauge (w.g.) must be maintained, and 100 fpm should be maintained through a glove port, if a glove is accidentally removed.