University of South Carolina

BIOSAFETY MANUAL

(Modified for USC Upstate)
FOREWORD
This manual is issued as a means of providing users of biohazards with information on the biosafety policies and procedures of University of South Carolina. These policies and procedures apply to work involving insertion of recombinant DNA into cells or organisms and to the use of agents of known or potential pathogenicity to humans, animals and plants. The responsibility placed on the University to provide a workplace that is free from recognized hazards makes it mandatory that all departmental activities conform to the intent of this manual.

The Institutional Biosafety Committee establishes University biosafety policy and reviews proposals to use materials that are designated as biohazards. The committee provides consultation and service necessary to ensure that the Office of Environmental Health and Safety (EHS) provides proper protection and adherence to the guides contained in this document. All users of designated biohazards must be familiar with the requirements set forth in this manual, and conduct their operations in accordance with them.

Acknowledgment
The University of South Carolina’s manual was originally modified from the University of East Carolina’s Biosafety Manual. This version, which is suitable for the Upstate campus of the USC system, was modified from the University of South Carolina’s version.
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ADMINISTRATION

Purpose of this Manual

Any work performed at the University of South Carolina involving recombinant DNA inserted into cells or organisms or involving agents of known or potential pathogenicity for humans, animals or plants must be conducted in a manner that affords protection to workers, animals, and the surrounding community. The biosafety program at the University of South Carolina has been established to ensure that adequate administrative and operational protection measures are in place. The University of South Carolina Biosafety Program, which consists of the Institutional Biosafety Committee (IBC) and the Office of Environmental Health & Safety (EHS), provides this manual in order to:

• Define the responsibilities of all parties involved in obtaining and using biohazards at the University of South Carolina.
• Advise all individuals working with biohazards of their rights and responsibilities under Federal and State laws.
• Provide the worker with a reference so as to assist in the safe handling of pathogenic agents.
• Provide information on the proper treatment and disposal of wastes.
• State the steps to be taken in the case of spills or other emergencies.

This manual is not intended for use as a guide for the control and prevention of person-to-person or patient-to-health care worker infections in human clinical activities. The Biosafety Program applies to research and teaching laboratories.
**Organization/Policy**

Whenever recombinant DNA or pathogenic agents are used at an institution, the safety of personnel and the general public becomes a prime consideration. The Institutional Biosafety Committee (IBC) and the Office of Environmental Health and Safety (EHS) have been charged with providing a quality biosafety program for the University. The IBC is authorized by the President to review and approve proposals and laboratory activities that utilize biohazardous agents. The Office of Environmental Health and Safety provides for the daily oversight of the biosafety program. The organizational structure of the biosafety program is shown below. Each component of the organization is charged with specific responsibilities in order to achieve the best possible worker protection, while producing minimal interference with research.

Where unsafe practices involving the use of biohazards or actions in violation of established guidelines are observed, the Biosafety Officer (BSO) has the authority to require suspension of the work until a thorough review can be made by the Institutional Biosafety Committee. If the Committee, at any time, is not satisfied with the adequacy of the biosafety practices employed in a project, they may require all work involving the agent to be suspended until satisfactory procedures have been adopted.

**Responsibilities**

**Institutional Biosafety Committee**

The Institutional Biosafety Committee is comprised of a minimum of five members. Membership selection is based on previous work experience and education so that the collective committee possesses knowledge of recombinant DNA technology, biosafety, physical containment techniques, institutional policies, applicable laws and standards, professional conduct and community standards. The committee meets on a regular basis, at least quarterly.

The Institutional Biosafety Committee is responsible for the administrative oversight of activities involving biological agents at USC. These responsibilities include, but are not limited to:

1. Develop policies and guidelines for biosafety at the University of South Carolina, including an annual review of the USC Biosafety Manual.
2. Review for approval all applications for the use of recombinant DNA or select agents.
3. Approve emergency plans for the containment and resolution of accidental spills and other related emergencies with an emphasis on risk reduction, personnel protection, and environmental protection.
4. As an agent of the Institution, assure the biosafety training of, as well as evaluate the experience of, Principal Investigators (PIs) and associated staff utilizing potentially
dangerous biological agents in research. The Institutional Biosafety Committee can refuse, suspend, or cancel authorization to use biological agents when training and/or experience is deemed inadequate.

5. Advise and provide technical expertise on matters regarding biosafety to the Biosafety Officer or other members of the USC community.

6. Conduct investigations of serious violations or problems and make recommendations to the Vice President for Research and other appropriate university administrators for the resolution of continued non-compliance or serious infractions. The Institutional Biosafety Committee can refuse, suspend, or cancel authorization to use biological agents in the event of continued non-compliance or serious infractions.

7. Review all instances of alleged infractions of the use of bio-hazardous agents or safety rules with the Biosafety Officer and responsible personnel and take necessary steps to correct such infractions.

8. Review plans for all new buildings and existing building modifications where bio-hazardous agents may be used.

9. Ensure that with regard to biosafety, all guidelines and standards from the National Institutes of Health (NIH), Centers for Disease Control (CDC) and United States Department of Agriculture (USDA) are reasonably met.

**Biosafety Officer**
The Biosafety Officer (BSO) is responsible for daily management of the biosafety program. Specific responsibilities include:

1. Conduct periodic assessments of laboratories to ensure compliance with regulatory requirements and university policies.

2. Investigate laboratory accidents and report problems, violations, and injuries or illnesses associated with biological agents, to the Institutional Biosafety Committee.

3. Develop and implement emergency plans for handling accidental spills and personnel contamination.

4. Provide advice and assistance to the Institutional Biosafety Committee and Principal Investigators concerning containment procedures and practices, laboratory security, recommended laboratory containment equipment, regulatory requirements, prudent practices, and other matters as appropriate.

5. Provide general biosafety training.


**Principal Investigator**
The Principal Investigator is the individual who submits the application to employ biohazardous agents in his or her work. This individual is fully responsible for adherence to all guidelines and regulations. The Principal Investigator is also fully responsible for the safe use of such agents by himself/herself and those under his or her direction.
Responsibilities of the Principal Investigator:

- Limit personnel, student, employee, and visitor exposure to biohazards to the lowest practical level, with special safety considerations for any individuals under 18 years of age in the lab (see below).
- Develop an individualized Laboratory Biosafety Plan for Biohazards, placing a priority on engineering and work practice controls to eliminate or minimize employee exposure.
- Submit the initial research application (and subsequent changes) to the Institutional Biosafety Committee for review. No research involving biohazards may be initiated until the research or proposed modification has been approved by the Institutional Biosafety Committee.
- Obtain Institutional Biosafety Committee approval for all rDNA and select agent projects involving animals before submission of protocol to the Institutional Animal Care and Use Committee.
- Personally train or arrange for the training of all personnel within 10 days of appointment with regard to the specific safety techniques and practices to be used with biohazards in the laboratory. Each person's proficiency with these tasks must be demonstrated to the Principal Investigator prior to working with the biohazardous agent. The Principal Investigator is also responsible for verifying each person's continued proficiency in applicable biosafety practices.
- Arrange and document the training of students and employees regarding biosafety procedures in the laboratory including routine and emergency procedures.
- Provide current posting and labeling of laboratories, stock materials, and associated equipment that may be contaminated.
- Provide adequate personal protective equipment and instruction on its proper use.
- Ensure that biohazardous wastes are properly prepared for disposal.
- Adhere to Institutional Biosafety Committee approved emergency plans in the event of accidental spills and/or personal contamination.
- Report immediately to EHS any suspected personnel exposures, theft of material, or other incidents regarding biosafety.
- Comply with shipping requirements for biohazardous materials.
- Submit information to NIH if required by guidelines. Obtain Institutional Biosafety Committee concurrence if direct communication to NIH is required.

Individual

The individual worker is the person who deals with the biohazardous agent on a regular basis and must be familiar with the potential hazards and requisite safety procedures associated with the agent.

Responsibilities of the Individual:

- Keep his or her exposure to the biohazardous agent as low as practical.
- Follow the Laboratory Biosafety Plan. Have a working knowledge of relevant emergency and decontamination procedures. Properly dispose of all biohazardous wastes.
• Assist the Principal Investigator in keeping all postings and labels of laboratories, materials, and equipment current.
• Report immediately to the Principal Investigator all suspected personnel exposures, theft of material, and any other biohazard related accidents. If the Principal Investigator is not available, the report should be made to EHS/Biosafety Officer.

Training
This manual does not provide detailed training regarding specific laboratory tasks and their associated biosafety procedures. Instruction concerning individual laboratory procedures and the development of a Laboratory Biosafety Plan are the responsibility of the Principal Investigator. EHS provides general training in the area of biosafety.

Application Procedures
Various governmental agencies are involved in controlling the use of biohazards. Governmental controls exist in the form of both recommendations and regulations. To ensure compliance with the various administrative control measures, the University of South Carolina has established formalized procedures for obtaining approval to use biohazards. Principal investigators are required to develop a Laboratory Biosafety Plan that describes the safety measures that will be employed in the workplace.

An Institutional Biosafety (Recombinant DNA) Application form must be submitted to the Biosafety Committee for any work with recombinant DNA or select agents. Three principal factors are considered by the Institutional Biosafety Committee in evaluating the adequacy of the safety provisions in a proposed usage: 1) the ability and experience of the applicant to cope with the hazards involved in the application, 2) the adequacy of the facilities and equipment for the proposed usage, and 3) the thoroughness and attention given to the safety precautions applied to the proposed experimental procedures. The Committee may specify further precautions for certain types of operations and particular projects. To assist in observing safety precautions and to satisfy itself that adequate measures of safety are being practiced, the Institutional Biosafety Committee directs the Biosafety Officer to serve as a liaison between the Committee and the individual researchers. Applications are approved for a period of three years, after which a new application must be submitted. Principal Investigators wishing to modify an approved recombinant DNA application must submit a revised rDNA-Application (in whole or just the revised section) to the IBC administrator (IBC@gwm.sc.edu). EHS will notify the Chair of the revision, who will determine if a full committee review and approval is warranted. Minor revisions may be approved by the Chair without a full committee review, but must be reported at the next convened IBC meeting.

Authorization to use recombinant DNA or other biohazards can be suspended or cancelled by the IBC when sufficient cause exists. Examples of sufficient cause include: serious or repeated failure to comply with NIH, CDC or other applicable regulations; repeated failure to comply with the USC Biosafety Manual, USC policies, and recognized good biosafety practices; or repeated failure to submit required documentation in a timely manner.
Laboratory Inspections

The staff of the EHS will conduct routine inspections of all work areas using biohazards. These routine inspections are intended to serve as review of the practices, procedures, and equipment in place in the workplace.

Safety violations discovered during routine inspections must be corrected within two weeks of the inspection. When correction of safety violations is not possible or practical within the two-week period, the principal investigator must provide a corrective action plan to EHS. This action plan must 1) be submitted within two weeks of the original inspection, 2) contain a time-table for correcting the safety violations and 3) be acceptable to the Biosafety Officer. A reinspection of the laboratory may be performed to verify that the corrections have been made. Failure to correct safety violations may be viewed by the Institutional Biosafety Committee as sufficient cause to suspend or cancel authorization to use recombinant DNA or other biohazards at the University of South Carolina.
GENERAL INFORMATION & PROCEDURES

Biohazard Definition
For purposes of the USC Biosafety Program, biohazards (biohazardous agents) are defined as 1) microorganisms (bacteria, fungi, viruses, Rickettsiae, Chlamydiae and parasites), cells, plants or animals into which recombinant DNA has been inserted, 2) microorganisms or other agents that are actual or potential pathogens for humans, animals or plants, and 3) select agents or toxins identified by the U.S. Departments of Health and Human Services (HHS) and of Agriculture (USDA).

NOTE: Although not covered in this manual, research involving human or other primate tissues and body fluids must also be registered with the Office of Environmental Health and Safety. Individuals potentially exposed to blood borne pathogens should be enrolled with the USC Occupational Health Services. The reader is also referred to the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories Appendix H.

Classification of Biohazards by Risk Group
The investigator must make an initial risk assessment based on the Risk Group of an agent. Agents are classified into four Risk Groups according to their relative pathogenicity for healthy adult humans by the following criteria:

- **Risk Group 1** agents are not associated with disease in healthy adult humans.
- **Risk Group 2** agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available.
- **Risk Group 3** agents are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk).
- **Risk Group 4** agents are 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).

The *NIH Guidelines for Research Involving Recombinant DNA Molecules* contains a comprehensive list of agents classified by hazard level. Also see CDC/NIH Biosafety in Microbiological and Biomedical Laboratories Section VII for additional information. Hyperlinks to both documents may be found in the References section of this manual.

Biosafety Levels
Four biosafety levels have been designated in the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories and in the *NIH Guidelines for Research Involving Recombinant DNA Molecules* Appendix G, which give detailed requirements for each level. The levels have been established based on the Risk Group of the infectious agent and the activities to be performed. It is important to note that the Principal Investigator is responsible for selecting and applying the recommended biosafety level for the work conducted. The investigator's unique knowledge and
judgment of the agent to be used is critical in assessing the associated risks of exposure. Each biosafety level consists of a combination of proscribed laboratory procedures and safety equipment:

**Biosafety Level 1:** The practices and equipment utilized in a Biosafety Level 1 facility are appropriate for work with defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans. Examples of these types of microorganisms include *Bacillus subtilis*, and infectious canine hepatitis. The criteria for Biosafety Level 1 are given in the Appendix.

**Biosafety Level 2:** The equipment, practices, and facilities used in Biosafety Level 2 laboratories are established for a broad range of indigenous moderate risk agents. Examples include the salmonellae, and *Toxoplasma* sp. The primary hazards to workers associated with these agents are accidental auto-inoculation, ingestion, and skin and mucous membrane exposure. Processes possessing the ability to produce aerosols must be conducted in primary containment devices. The criteria for Biosafety Level 2 are given in the Appendix.

**Biosafety Level 3:** Biosafety Level 3 facilities are established for the use of indigenous or exotic agents that possess the potential for infection by aerosol, and the results of such infection may have serious or lethal consequences. Typical examples of agents designated as requiring Biosafety Level 3 facilities include *Mycobacterium tuberculosis*, St. Louis encephalitis virus, and *Coxiella burnetii*.

**Biosafety Level 4:** Level 4 facilities are designed for work with dangerous and exotic agents, which pose a high risk to individuals to contact a life-threatening disease. In these facilities, all manipulations are considered to be of high risk, and the procedures and safety equipment are designed to prevent exposure.

Criteria for Biosafety Levels on the following types of research may be found in the referenced sections of CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* and *NIH Guidelines for Research Involving Recombinant DNA Molecules*.

- Large-scale uses of organisms containing recombinant DNA molecules, *NIH Guidelines Appendix K*
- Recombinant DNA research involving animals, *BMBL Section IV and NIH Guidelines Appendix Q*. The criteria for Animal Biosafety Levels 1 and 2 are given in the Appendix.
- Recombinant DNA research involving plants, *NIH Guidelines Appendix P*; for additional information on containment issues, see *A Practical Guide to Containment: Greenhouse Research with Transgenic Plants and Microbes*

The Institutional Biosafety Committee may lower the containment levels for certain experiments, if deemed appropriate. Lowered biosafety levels may be requested for the following uses:

- HIV-derived lentivirus vectors that are designed to be self-inactivating and that are pseudotyped with a non-HIV envelope – Biosafety Level 2.
Adeno-associated virus vectors produced with adenovirus-free packaging systems or oncoretrovirus vectors pseudotyped with an ecotropic (e.g., mouse-specific) envelope – Biosafety Level 1.

Viral vector stocks proven to contain no replication-competent virus – Biosafety Level 1. Note that each vector stock produced must be tested with an appropriate assay at a limit of sensitivity of 1 infectious unit per milliliter. Vectors included in this category:
- Adeno-associated virus vectors.
- Self-inactivating lentivirus vectors pseudotyped with a non-HIV envelope.
- Oncoretrovirus vectors pseudotyped with envelopes other than ecotropic.
- Herpes virus amplicon vectors.
- “Gutless” or similar adenoviral vectors containing less than \( \frac{2}{3} \) of the wild-type genome. Vector stocks should be tested at a limit of sensitivity of 1 in \( 10^6 \) particles compared to a known positive control.

**Bioaerosols**

Bioaerosols are microorganisms dispersed or spread in the form of aerosols or droplets. They are in either dry or liquid forms, typically 5μm in diameter. Due to their small size, aerosols do not settle quickly and can remain airborne for long periods of time. If inhaled, bioaerosols can be carried to the alveoli, potentially causing infection. Sputum and other clinical specimens submitted for culture may contain unsuspected microorganisms, such as mycobacterium, which are highly infectious by the airborne route. If generation of an aerosol is likely to occur during the processing of these specimens, the use of a biosafety cabinet is recommended. Aerosols can be generated by many common laboratory techniques and can represent a significant source of laboratory-acquired infections. Almost any handling of liquids or of dry powders is likely to generate aerosols and droplets. Examples of procedures generating aerosols from laboratory equipment include, but are not limited to: high speed blending, grinding, filtering, mixing, sonicating, agitation, flaming, pipetting, opening vials of lyophilized cultures, shaking and centrifuging.

The careful performance of certain laboratory procedures must be emphasized because some procedures can generate small particle aerosols. Those procedures, when used with highly infectious microorganisms or toxins should be confined to biosafety cabinets or hoods. To minimize aerosol production, pipettes should be drained gently with the tip against the inner wall of the receiving tube or vessel. Do not expel infectious materials forcibly from pipettes, and never bubble air through a suspension of infectious agents in open containers. It is recommended that these procedures be conducted in a biosafety cabinet when Biosafety Level 3 precautions are being used. The equipment should be selected for features designed to contain infectious liquids or aerosols.

Centrifuges with sealed buckets, safety trunnion cups, or sealed heads are effective in preventing escape of liquids and aerosols. In addition, sealed centrifuge tubes or bottles should be used. If fluid should escape from a cup or rotor during high-speed operation, the potential for extensive contamination and multiple infections is great. Centrifuge tubes, bottles, rotors and safety trunnion cups should be visually inspected on a regular basis to ensure that leakage does not
occur during operational procedures. HEPA filters should be installed between the chamber and the vacuum pump of the ultracentrifuge.

Improper technique in the flaming of inoculating loops can also result in the spread of infectious agents. Spatter and release of droplets or aerosols can be prevented by heating the shaft until the sample has been heat-dried before flaming the loop itself. Spatter can also be controlled effectively by using a die-arm burner or electric microincinerator. The process of flaming can be avoided by using sterile, disposable loops.

**Dangers of Cell and Tissue Culture Systems**

Many biochemistry, physiology, microbiology, and cancer research laboratories use cell cultures as routine source materials. The actual hazards of this work are not clearly recognized and may be minimal, with certain exceptions. Some hazards may involve diseases that develop slowly over many years, e.g. solid tumors or degenerative neurological diseases. Handling procedures for cell cultures, therefore, must cause the least interference with the experimental work, but provide personnel protection consistent with the presumed hazards. Most cell cultures are known to harbor viruses, either adventitiously or deliberately. In these cases, the appropriate procedures for the known or presumed virus should be used with the cell culture. Primary and permanent cell lines from mice, hamsters, humans, and rats should be handled as if they carry low risk infections. Human isolates from malignant tissues or those from tissues susceptible to or likely to harbor mammalian oncogenic viruses should be considered as moderate risk agents. Cells from Herpes and Epstein-Barr virus-transformed cultures should be handled as moderate risk viruses. All established or permanent cultures of human lymphocytes should be handled on the assumption they harbor a moderate risk agent. Under no conditions should an individual handle lymphoid cells of a line derived from him- or herself, or first-degree relative. The 293 and 293T cell lines often used for production of viral vectors require BSL-2 procedures.
DETAILED REQUIREMENTS FOR WORK WITH BIOHAZARDS

Warning Signs and Postings

The universally accepted biohazard warning symbol shall be used throughout the institution to notify workers about the presence of biohazardous agents. It is the responsibility of the principal investigator to ensure that all necessary postings are installed and properly maintained. The warning symbol must be removed when the hazardous agent is no longer in use or present. The biohazard symbol included on postings should be orange or red in color with a contrasting background. As a general rule, the location of the posting is predicated by how access is gained to the biohazard area. In most cases, the door to any laboratory containing a designated biohazardous agent should be posted. In addition, postings should also be displayed in other areas such as biosafety cabinets, freezers, or other specially designated work and storage areas.

Universal biohazard labels must be affixed to containers of regulated waste, refrigerators and freezers containing blood or other infectious materials. Labels must be affixed to other containers used to store, transport, or ship blood or other potentially infectious materials. All individual containers of biohazardous agents should also be labeled to identify the content and any special precautionary measures that should be taken. Red bags or red containers may be substituted for this labeling requirement.

Laboratory Biosafety Plans

A written Laboratory Biosafety Plan is required for each research and teaching laboratory wherein employees and students may be exposed to biohazards, including exposures that could result from work with infectious microorganisms and recombinant DNA molecules. The Laboratory Biosafety Plan will address routine specific safety precautions for the laboratory, and specify correct responses to accidents that might occur in the area. The principal investigator is responsible for the safety of workers and visitors in the laboratory and, therefore, is responsible for development of the plan and for ensuring compliance with safe practices. The Laboratory Biosafety Plan should be written with reference to CDC/NIH Biosafety in Microbiological and Biomedical Laboratories and NIH Guidelines for Research Involving Recombinant DNA Molecules. The WHO Laboratory Biosafety Manual also contains helpful information on laboratory biosafety.

The Biosafety Officer and/or the Institutional Biosafety Committee will review the individual Laboratory Biosafety Plan periodically. All employees and students working in a research or teaching laboratory with potential exposure to biohazards will be appropriately trained within ten days of initial employment and annually thereafter. The principal investigator (PI) is responsible for providing this training, or for ensuring attendance by the worker at appropriate safety training sessions. The PI will document training by maintaining records of attendance. Where appropriate, employees and students that work in laboratories with infectious or potentially infectious materials will be enrolled in the occupational health program.
**Biosafety Cabinets**

Biological safety cabinets are used for the protection of personnel from aerosols produced by experimental procedures involving etiological agents. When used along with proper microbiological techniques, they provide an effective containment system for the manipulation of Biosafety Level 2 and 3 agents. Although biosafety cabinets are designed to prevent the escape of aerosols, personnel must have appropriate training in their safe use. Rapid movements in front of the cabinet, slamming of doors, or brisk walking can drastically change the airflow patterns, resulting in the escape of aerosols. *Clean air horizontal laminar flow workbenches do not operate like biosafety cabinets.* The primary purpose of a clean air workbench is to protect the material in the air stream, not the worker. Filtered air is blown horizontally across the material in the hood, towards the worker. Clean air hoods should never be used to manipulate pathogenic microorganisms, human tissues or animal tissues.

Biosafety Cabinet selection should be based on:
- 1. Hazard classification
- 2. Amount of protection needed for research products or personnel
- 3. Amount of hazardous aerosols generated

There are essentially three types of Biosafety Cabinets: Class I, II and III. (See *BMBL Appendix A* for more information.)

**Class I:** This cabinet provides partial containment of aerosols, but no protection for the material or experiment in the cabinet. Although the cabinet provides adequate personnel protection, cross contamination may result from unfiltered air flowing over the work area. The front opening of the cabinet should be approximately 8 inches in height. Air velocity should be a minimum of 75 linear feet per minute (lfpm). Class I cabinets are not recommended for use with infectious agents.

**Class II:** Both the worker and the material in the cabinet are protected with a Class II cabinet. Etiologic agents, recombinant DNA molecules, and moderate risk oncogenic viruses may be manipulated safely in Class II cabinets. Class II cabinets have the following characteristics:
- • High efficiency particulate air filter (HEPA) on the intake
- • Open front access
- • Downward clean airflow
- • Exhaust air HEPA filtered

**Class IIA** cabinets recirculate 70% of the total air inside the hood. Flammable solvents, toxic chemicals, and radioactive materials should not be used in a class IIA cabinet.

**Class IIB** cabinets are hard-ducted to the building exhaust system and contain negative pressure plena. These features, plus a face velocity of 100 lfpm, allow work to be done with toxic chemicals or radionuclides.

**Class III** cabinets provide the highest level of personnel protection. The entire unit is enclosed, thereby preventing agent from contacting the worker. Class III cabinets are self-contained, closed front, stainless steel, and are operated at negative pressure. Manipulations are performed with
arm length rubber gloves sealed to the front of the unit. Highly infectious agents can be manipulated safely in Class III cabinets.

**Certification** When pathogenic materials are to be used in a biosafety cabinet, a qualified individual must certify the unit. Cabinets require certification as follows:
1. Initial installment
2. Annually thereafter
3. After moving the cabinet
4. After HEPA filter replacement

If a cabinet is not certified or the user does not plan to use pathogenic material in the system, a notice should be affixed to the cabinet indicating: *"Cabinet not certified. Do not use pathogens."*

**Start up Work Practices with Biosafety Cabinets**
1. Turn off ultraviolet (UV) lamp, if one is used.
2. Turn on fluorescent light, and remove any obstructions and foreign materials that block the front and rear ventilation grills.
3. Adjust view screen to proper height.
4. Turn on blower and allow five minutes to purge the air.
5. Disinfect the interior surfaces of the biosafety cabinet by wiping down with disinfectant. Do not use sodium hypochlorite (household bleach, Chlorox) to disinfect biosafety cabinets because of potential corrosion.
6. Assemble all items for the experiment in the biosafety cabinet before initiating work and keep clean items segregated from dirty items. Work from "clean" to "dirty" areas.
7. Work at least six inches back from the front air intake grill. Do not block front or rear ventilation grills.
8. Avoid sudden movements and remove arms slowly from biosafety cabinets.
9. Operators should keep their face above the opening of the biosafety cabinet.

**Sterilization and Disinfection**
Sterilization is a method or process to remove all viable microorganisms from an object or material. The process must consistently produce objects that are negative to chemical and biological indicators of contamination. Achieving sterility depends on several factors including the number and type of organisms present, the temperature and the length of contact time. For further information see WHO Laboratory Biosafety Manual Chapter 14.

**Autoclaving** Steam sterilization (autoclaving) will kill most microorganisms when steam under pressure is applied at 121°C for a minimum of 15 minutes. It is important to remember that sterilization will not be complete if steam does not reach all surfaces of the object. This is of particular concern for items that have a high soil load and densely packed materials. Spore strips can be placed at the center of the pack as a biological indicator of sterility. Autoclave tape is not an indicator of sterility; it simply indicates that the proper temperature has been achieved on the surface. Sterilization is not practical for tables, cabinets, and some equipment, so disinfection must be utilized. The term disinfection implies the use of antimicrobial chemicals on inanimate objects with the purpose of destroying all non-spore forming organisms of pathogenic nature or which would compromise the integrity of the experiment. Note that disinfection does not mean the destruction of all organisms.
Chemical disinfectants Disinfectants destroy microorganisms by coagulating or denaturing proteins, injuring cell membranes and stopping normal enzymatic reactions. The range of susceptibility of microorganisms to disinfectants is relatively broad. A table summarizing the properties of the most commonly recommended chemical disinfectants is included below. The vegetative bacteria, fungi, and lipid-containing viruses are highly susceptible to all of the agents listed. Non-lipid containing viruses are moderately resistant to these agents, while spore forms are the most resistant.

### Chemical Disinfectants

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>Commonly Available Preparations</th>
<th>Effective Concentrations of Active Agents</th>
<th>Applications</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorine compounds</td>
<td>5.25% sodium hypochlorite <em>(household bleach)</em></td>
<td>0.5% (1:10 dilution, 5000 ppm); <em>solution stable if made with deionized water and protected from heat and light</em></td>
<td>Biohazard spills, contaminated instruments and glassware, liquid waste</td>
<td>-Broad spectrum with activity against non-lipid viruses, some bacterial spores</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.05% (1:100 dilution, 500 ppm); <em>solution unstable, make fresh dilutions as needed</em></td>
<td>Cleaned work surfaces; avoid use in biosafety cabinets</td>
<td>-Highly reactive with organic matter; evolution of chlorine gas</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-For liquid waste add to a final conc. of 0.5% in a chemical fume hood</td>
</tr>
<tr>
<td>Alcohols (ethyl or isopropyl)</td>
<td>95-100%</td>
<td>70-90%</td>
<td>Work surfaces, equipment surfaces, animal injection sites</td>
<td>-Flammable</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>-Activity reduced by presence of organic matter</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>Various concentrates and ready-to-use solutions or sprays <em>(‘Lysol’ concentrate is 5-6% o-benzyl-p-chlorophenol)</em></td>
<td>0.2-3%</td>
<td>Biohazard spills, contaminated instruments and glassware, liquid waste</td>
<td>-Dilute with deionized water</td>
</tr>
<tr>
<td></td>
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<td>-For liquid waste add to a final conc. of at least 1%</td>
</tr>
<tr>
<td>Quaternary ammonium compounds</td>
<td>Various concentrates and ready-to-use solutions or sprays <em>(benzylalkonium chloride, Hyamine 3500)</em></td>
<td>0.1-2%</td>
<td>Work surfaces, equipment surfaces, contaminated glassware</td>
<td>-Often mixed with alcohols</td>
</tr>
<tr>
<td></td>
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<td>-- Dilute with deionized water</td>
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<td>-Activity reduced by presence of organic matter and anionic detergents</td>
</tr>
</tbody>
</table>

N.B.: All agents may require up to 30 min contact time
Equipment labeled with the universal biohazard symbol must be thoroughly disinfected with a 1:10 dilution of bleach or other EPA approved disinfectant before sending offsite for surplus or repair. The biohazard symbol must be removed before sending equipment to surplus.

**Transportation and Shipment of Biological Materials**

Any movement or transport of biohazardous materials in a laboratory or within or between buildings at the University of South Carolina should be performed in such a manner as to prevent any spilling and/or leakage. Materials should be transported in containers that can be sealed in some manner. If the outside of the primary container is suspected of being contaminated, a secondary container should be used. If the material to be transported could puncture the primary container, a secondary, puncture-resistant container should be used. Any equipment that is suspected of being contaminated must also be contained or decontaminated prior to movement or service work.

Shipment of biohazards outside of the institution must conform to the applicable regulations outlined in the *NIH Guidelines* Appendix H and *BMBL* Appendix C. It should be especially noted that individuals responsible for shipping of biohazards must have documentation of training in accord with the applicable regulations.

**Personal Protective Equipment**

Personal protective clothing shall be worn in instances where engineering controls are not feasible but should not be used as a substitute for engineering controls. Individuals will be encouraged to use appropriate personal protective equipment as indicated by the principal investigator and/or EHS. Personal protective equipment provided at no cost and readily accessible at the worksite includes, but is not limited to the following: gloves, gowns, laboratory coats, face shields or masks, and eye protection. Accommodations will be made for individuals determined to be unable to use certain protective devices.

**Personal Protective Clothing**

It is the responsibility of the principal investigator or the department to provide employees with personal protective equipment. The principal investigator is also responsible for ensuring that the protective equipment is being used appropriately. Disposable protective clothing, including gloves, must be treated as biohazardous waste after use. Disposable single use gloves shall be replaced as soon as possible when visibly soiled, torn, punctured, or when their ability to function as a barrier is compromised. Disposable gloves shall never be washed or disinfected for reuse. Utility gloves may be disinfected for reuse if the integrity of the glove is not compromised. However, they must be discarded if they are cracked, peeling, discolored, torn, punctured, or exhibiting any sign of deterioration.

Masks and eye protection shall be worn whenever splashes, spray, droplets, or aerosols of potentially infectious materials may be generated and there is a potential for eye, nose, or mouth contamination. All personal protective equipment shall be removed before leaving the work area. When personal protective equipment is removed, it must be placed in an appropriately designated area or container for storage, washing, decontamination, or disposal.
Housekeeping

All equipment and working surfaces will be cleaned and decontaminated upon completion of procedures, spills, or after contact with blood or other potentially infectious materials using an approved disinfectant. Protective coverings, such as absorbent paper, are to be removed and replaced when overtly contaminated or at completion of procedures. All receptacles intended for reuse, such as bins, pails, or cans that may be contaminated should be inspected and decontaminated on a regular basis. Broken glassware will be cleaned up using mechanical means, such as brush, broom, dustpans, tongs, forceps, etc. Equipment which may become contaminated with blood other potentially infectious materials shall be checked routinely and prior to servicing or shipping and shall be decontaminated as necessary. Laboratory personnel must disinfect area daily. If an area becomes contaminated with blood or body fluids, the excess fluid shall be absorbed with disposable absorbent material and placed in a biohazard container or bag. Decontamination of contaminated surfaces must follow using an EPA approved disinfectant. Gloves must be worn throughout the entire procedure.

Biohazardous Waste

Sharps

The use of sharps with biohazards should be avoided if at all possible. Plastic containers should be substituted for glass containers whenever possible.

Sharps should be disposed of into puncture-resistant, closable, leak proof, labeled, or color-coded containers. Sharps containers should be easily accessible to personnel and located as close as feasible to the work area. Containers should remain upright and not overfilled. Used needles shall not be recapped or sheared, but should be deposited whole into the appropriate sharps containers. The following items should always be placed in approved sharps containers. They should never be placed in biohazardous waste containers.

- Ampules
- Scalpels
- Capillary Tubes
- Needles
- Microtome Blades
- Capillary Pipettes
- Pasteur Pipettes
- Microscopy slides
- Butterfly Units
- Razor Blades
- Cover Slips

Regulated Waste

All contaminated or potentially contaminated materials will be placed in biohazard bags/containers that are closable, leak proof, labeled, or color-coded. This waste should be closed prior to removal. If any container becomes contaminated, it shall be placed in a secondary container meeting the same standards. Call Lab Manager (503-5921) for proper disposal. Only waste designated as "biohazardous" should be put into designated biohazardous waste containers.

Waste Always Considered Biohazardous

- Cultures and stocks of infectious agents or cells/organisms containing recombinant DNA
- Culture dishes and all materials that have come in contact with infectious agents or biohazards
- Organs/Tissues derived from animals or plants exposed to biohazards
- Gloves and disposable personnel protective clothing
**Pregnancy**

Any female or male working with biohazards who knows, suspects, or is trying to become pregnant or trying to father a child should contact EHS as soon as possible to obtain information on pregnancy and the risks from biohazards. A confidential meeting will be scheduled. All users of biohazards will be informed of the special risks associated with reproduction and biohazardous agent exposure. This information will be communicated to the individuals during the confidential meeting with the individual. It is up to the mother to compare the benefits of her employment against the possible risks involving occupational exposure to biohazards to a known or potential unborn child. The mother should know that the Pregnancy Discrimination Act, an amendment of Title VII of the Civil Rights Act of 1964, states that "women affected by pregnancy, childbirth, or related medical conditions shall be treated the same for all employment related purposes, including the receipt of benefits under the fringe benefits programs, as other persons not so affected but similar in their ability or inability to work." In addition, the Equal Employment Opportunity Commission (a Federal Agency) is responsible for examining cases for compliance with this act. Exposure to mutagenic agents would be of concern to fertile employees of either sex, and such exposure should be minimized. Diagnostic microbiologists, serologists, and chemistry laboratory workers, who have direct contact with patients with infectious diseases, may be exposed unknowingly to a variety of infectious agent in the specimens that they process (i.e. rubella virus, hepatitis B virus, enteroviruses, herpes simplex virus, varicella virus, Treponema pallidum (syphilis), and toxoplasma). The concern for the pregnant employee may be increased if she is handling viruses requiring Biosafety Level 3 or 4 safety precautions for which the effects of maternal infection on the fetus are unknown.

**Minors**

In some instances, individuals under the age of 18 may encounter situations where exposure to biohazards may be involved. Exposure to biohazards of those under 18 years of age require special attention because, from a regulatory standpoint, these individuals are considered to be minors. Most exposures to biohazards are the result of teaching laboratories or advanced placement summer science learning programs. Principal investigators with minors under their direction must be aware of the following special provisions established for the safety of individuals less than 18 years of age.

It is the responsibility of the principal investigator to fully adhere to all regulations applicable to the safe use of any biohazards under their direction, with special attention given to the consideration of minors. No minor shall be permitted to work with open containers or dispersable forms of biohazards agents. It is strongly recommended that minors not work directly with any sources of biohazards, if at all possible. No minor shall work in the vicinity of any source of biohazards without the immediate and constant supervision of an adult who is familiar with all applicable safety practices.
EXPOSURES AND EMERGENCIES

Emergency Phone Numbers

For USC Upstate:
Emergency (Fire, Rescue, and Police) 503-5911
Lab Manager 503-5921
Risk Manager 503-5905

For USC Columbia:
Emergency (Fire, Rescue, and Police) 777-9111
USC Environmental Health & Safety 777-5269

Cleanup Guidelines

Small Biohazardous Spill
1. Appropriate personal protective clothing, such as gloves and a lab coat, should be worn when cleaning up biohazardous spills. Additional personal protective clothing must be worn, such as splash goggles, fluid resistant body protection, face shield, and respiratory protection, depending on the biosafety level.
2. Paper towels or other absorbent materials soaked with an appropriate disinfectant should be placed over liquid spills, to absorb any excess fluid. Discard these absorbent materials into a biohazardous waste container.
3. Pour an appropriate disinfectant solution (1:10 dilution of household bleach) slowly to the outer margin of the spill and allow it to flow in, using care not to cause spatter. This method helps to minimize aerosolization.
4. Let stand for 20 to 30 minutes to allow adequate contact time and clean up with more paper towels.
5. Discard materials (paper towels, gloves, and other wastes from clean-up) into an autoclave bag and autoclave.
6. Wash hands for at least ten seconds with mild antibacterial soap and water.

Large Biohazardous Spill
1. Hold your breath, leave the room immediately, and close the door.
2. Notify supervisor and warn others not to enter the contaminated area. Post a temporary warning sign.
3. Remove and put contaminated garments into a container for autoclaving and thoroughly wash your hands and face with mild antibacterial soap and water.
4. Wait 30 minutes to allow dissipation of spill-created aerosols by the room ventilation air changes.
5. Appropriate personal protective equipment, such as lab coats, masks, and double gloves, should be worn before reentering the room. Additional personal protective clothing must be worn, such as splash goggles, fluid resistant body protection, face shield, and respiratory protection, depending on the biosafety level.
6. Paper towels or other absorbent materials soaked with an appropriate disinfectant should be placed over liquid spills, to absorb any excess fluid. Discard these absorbent materials into a biohazardous waste container.
7. Pour an appropriate disinfectant solution (1:10 dilution of household bleach) slowly to the outer margin of the spill and allow it to flow in. To minimize aerosolization, avoid pouring the disinfectant solution directly onto the spill.
8. Let stand 20 to 30 minutes to allow an adequate contact time.
9. Using an autoclavable dust pan, squeegee, and forceps for sharp materials, transfer all contaminated materials (paper towels, glass, liquid, gloves, etc.) into an autoclave bag and autoclave according to standard directions.
10. Wash and mop adjacent area and spill area with an appropriate disinfectant-detergent solution.
11. Remove and discard protective clothing. Shower and wash with antibacterial soap.
12. Notify Lab Manager.

Biohazardous Spill Inside a Biosafety Cabinet
Chemical decontamination procedures should be initiated at once while the cabinet continues to operate to prevent escape of contaminants from the cabinet. Be careful with paper towels, which can be sucked into the blower fan or HEPA filters.
1. Spray or wipe walls, work surfaces, and equipment with an appropriate disinfectant (e.g. 70% alcohol). Individuals should wear gloves during this procedure.
2. If cabinet is Class II, flood the top work surface tray and the drain pan below the work surface with a disinfectant and allow to stand for at least 20 minutes.
3. Wipe excess disinfectant from the tray. For Class II cabinets, drain the tray into the cabinet drain pan, lift out tray and removable exhaust grillwork, and wipe off top and bottom (underside) surfaces with a disinfectant. Replace grillwork and drain disinfectant from the drain pan into an appropriate container and autoclave according to standard procedures. Gloves, cloths or sponges should be autoclaved and discarded into biohazardous waste containers.
4. Wash hands for at least ten seconds with mild antibacterial soap and water.
REFERENCES

*CDC/NIH Biosafety in Microbiological and Biomedical Laboratories*

*NIH Guidelines for Research Involving Recombinant DNA Molecules*

*Possession, Use and Transfer of Select Agents and Toxins*
http://www.cdc.gov/od/sap/pdfs/42_cfr_73_final_rule.pdf

*A Practical Guide to Containment: Greenhouse Research with Transgenic Plants and Microbes*
http://www.isb.vt.edu/greenhouse/green_man.intro.cfm

*World Health Organization Laboratory Biosafety Manual*
APPENDIX

As a convenience for individuals using biohazards the essential elements of BSL-1, BSL-2, ABSL-1 and ABSL-2 are summarized below (from the CDC /NIH Biosafety in Microbiological and Biomedical Laboratories).

**Biosafety Level 1 (BSL-1)**

**Biosafety Level 1** is suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. The laboratory is not necessarily separated from the general traffic patterns in the building. Work is generally conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is neither required nor 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.

The following standard and special practices, safety equipment and facilities apply to agents assigned to Biosafety Level 1:

A. **Standard Microbiological Practices**
   1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments or work with cultures and specimens are in progress.
   2. Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
   3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in the work areas. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.
   4. Mouth pipetting is prohibited; mechanical pipetting devices are used.
   5. Policies for the safe handling of sharps are instituted.
   6. All procedures are performed carefully to minimize the creation of splashes or aerosols.
   7. Work surfaces are decontaminated at least once a day and after any spill of viable material.
   8. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leakproof container and closed for transport from the laboratory. Materials to be decontaminated outside of the immediate laboratory are packaged in accordance with applicable local, state, and federal regulations before removal from the facility.
   9. A biohazard sign can be posted at the entrance to the laboratory whenever infectious agents are present. The sign may include the name of the agent(s) in use and the name and phone number of the investigator.
   10. An insect and rodent control program is in effect (see Appendix G).

B. **Special Practices** None

C. **Safety Equipment (Primary Barriers)**
   1. Special containment devices or equipment such as a biosafety cabinet are generally not required for manipulations of agents assigned to Biosafety Level 1.
   2. It is recommended that laboratory coats, gowns, or uniforms be worn to prevent contamination or soiling of street clothes.
3. Gloves should be worn if the skin on the hands is broken or if a rash is present. Alternatives to powdered latex gloves should be available.

4. Protective eyewear should be worn for conduct of procedures in which splashes of microorganisms or other hazardous materials is anticipated.

D. Laboratory Facilities (Secondary Barriers)
1. Laboratories should have doors for access control.
2. Each laboratory contains a sink for handwashing.
3. The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.
4. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surface and equipment.
5. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning.
6. If the laboratory has windows that open to the exterior, they are fitted with fly screens.

**Biosafety Level 2 (BSL-2)**

Biosafety Level 2 is similar to Biosafety Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs from BSL-1 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; (3) extreme precautions are taken with contaminated sharp items; and (4) certain procedures in which infectious aerosols or splashes may be created are conducted in biosafety cabinets or other physical containment equipment.

The following standard and special practices, safety equipment, and facilities apply to agents assigned to Biosafety Level 2:

A. Standard Microbiological Practices
1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.
2. Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.
4. Mouth pipetting is prohibited; mechanical pipetting devices are used.
5. Policies for the safe handling of sharps are instituted.
6. All procedures are performed carefully to minimize the creation of splashes or aerosols.
7. Work surfaces are decontaminated on completion of work or at the end of the day and after any spill or splash of viable material with disinfectants that are effective against the agents of concern.
8. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leakproof container and closed for transport from the laboratory. Materials to be decontaminated off-site from the facility are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.
9. An insect and rodent control program is in effect (see Appendix G).
B. Special Practices

1. Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents is in progress. In general, persons who are at increased risk of acquiring infection, or for whom infection may have serious consequences, are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at increased risk of acquiring infections. The laboratory director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory or animal room.

2. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazards and meet specific entry requirements (e.g., immunization) may enter the laboratory.

3. A biohazard sign must be posted on the entrance to the laboratory when etiologic agents are in use. Appropriate information to be posted includes the agent(s) in use, the biosafety level, the required immunizations, the investigator’s name and telephone number, any personal protective equipment that must be worn in the laboratory, and any procedures required for exiting the laboratory.

4. Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).

5. 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.

6. Biosafety procedures are incorporated into standard operating procedures or in a biosafety manual adopted or prepared specifically for the laboratory by the laboratory director. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

7. The laboratory director ensures that laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural or policy changes.

8. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.

   a. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.

   b. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

   c. Syringes which re-sheathe the needle, needleless systems, and other safety devices are used when appropriate.

   d. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal, according to any local, state, or federal regulations.

9. Cultures, tissues, specimens of body fluids, or potentially infectious wastes are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.
10. Laboratory equipment and work surfaces should be decontaminated with an effective disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated according to any local, state, or federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.

11. Spills and accidents that result in overt exposures to infectious materials are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

12. Animals not involved in the work being performed are not permitted in the lab.

C. Safety Equipment (Primary Barriers)

1. Properly maintained biosafety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are used whenever:
   a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or embryonate eggs.
   b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a biosafety cabinet.

2. Face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face when the microorganisms must be manipulated outside the BSC.

3. Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel.

4. Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate. Gloves are disposed of when overtly contaminated, and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Disposable gloves are not washed, reused, or used for touching "clean" surfaces (keyboards, telephones, etc.), and they should not be worn outside the lab. Alternatives to powdered latex gloves should be available. Hands are washed following removal of gloves.

D. Laboratory Facilities (Secondary Barriers)

1. Provide lockable doors for facilities that house restricted agents (as defined in 42 CFR 72.6).

2. Consider locating new laboratories away from public areas.

3. Each laboratory contains a sink for handwashing.

4. The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are inappropriate.

5. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.

6. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.

7. Install biosafety cabinets in such a manner that fluctuations of the room supply and exhaust air do not cause the biosafety cabinets to operate outside their parameters for containment. Locate
biosafety cabinets away from doors, from windows that can be opened, from heavily traveled laboratory areas, and from other potentially disruptive equipment so as to maintain the biosafety cabinets' air flow parameters for containment.

8. An eyewash station is readily available.

9. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

10. There are no specific ventilation requirements. 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. If the laboratory has windows that open to the exterior, they are fitted with fly screens.

**Animal Biosafety Level 1 (ABSL-1)**

Animal Biosafety Level 1 (ABSL-1) is suitable for work involving well characterized agents that are not known to cause disease in healthy adult humans, and that are of minimal potential hazard to laboratory personnel and the environment.

**A. Standard Practices**

1. The animal facility director establishes policies, procedures, and protocols for emergency situations. Each project is subject to pre-approval by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biohazard Committee (IBC). Any special practices are approved at this time.

2. Only those persons required for program or support purposes are authorized to enter the facility. Before entering, persons are advised of the potential biohazards and are instructed on the appropriate safeguards.

3. An appropriate medical surveillance program is in place.

4. A safety manual is prepared or adopted. Personnel are advised of special hazards, and are required to read and follow instructions on practices and procedures.

5. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use should only be done in designated areas and are not permitted in animal or procedure rooms.

6. All procedures are carefully performed to minimize the creation of aerosols or splatters.

7. Work surfaces are decontaminated after use or after any spill of viable materials.

8. All wastes from the animal room (including animal tissues, carcasses, and contaminated bedding) are transported from the animal room in leak-proof, covered containers for appropriate disposal in compliance with applicable institutional or local requirements. Incineration is recommended.

9. Policies for the safe handling of sharps are instituted.

10. Personnel wash their hands after handling cultures and animals, after removing gloves, and before leaving the animal facility.

11. A biohazard sign must be posted on the entrance to the animal room whenever infectious agents are present. The hazard warning sign identifies the infectious agent(s) in use, lists the name and telephone number of the responsible person(s), and indicates the special requirements for entering the animal room (e.g., the need for immunizations and respirators).

12. An insect and rodent control program is in effect (see Appendix G).

**B. Special Practices:** None.

**C. Safety Equipment (Primary Barriers):**
1. The wearing of laboratory coats, gowns, and/or uniforms in the facility is recommended. Laboratory coats remain in the animal room. Gowns and uniforms are not worn outside the facility.  
2. Persons having contact with non-human primates should assess their risk of mucous membrane exposure and wear appropriate eye and face protection.  

D. Facilities (Secondary Barriers)  
1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building.  
2. External facility doors are self-closing and self-locking. Doors to animal rooms open inward, are self-closing, and are kept closed when experimental animals are present. Cubicle room inner doors may open outward or be horizontal or vertical sliding.  
3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors, and ceilings) are water resistant.  
4. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas.  
5. Windows are not recommended. Any 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.  
6. If floor drains are provided, the traps are always filled with water and/or an appropriate disinfectant.  
7. Ventilation should be provided in accordance with the Guide for Care and Use of Laboratory Animals, latest edition. No recirculation of exhaust air should occur. It is recommended that animal rooms maintain negative pressure compared to adjoining hallways.  
8. The facility has a hand washing sink.  
9. Cages are washed manually or in a cage washer. The mechanical cage washer should have a final rinse temperature of at least 180°F.  
10. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.  

Animal Biosafety Level 2 (ABSL-2)  
Animal Biosafety Level 2 involves practices for work with those agents associated with human disease. It addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure. ABSL-2 builds upon the practices, procedures, containment equipment, and facility requirements of ABSL-1.  

A. Standard Practices  
1. Aside from the standard policies, procedures, and protocols for emergency situations established by the facility director, appropriate special policies and procedures should be developed as needed and approved by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biohazard Committee (IBC).  
2. Access to the animal room is limited to the fewest number of individuals possible. Personnel who must enter the room for program or service purposes when work is in progress are advised of the potential hazard.  
3. An appropriate medical surveillance program is in place. All personnel receive appropriate immunizations or tests for the agents handled or potentially present (e.g., hepatitis B vaccine, TB skin testing). When appropriate, a serum surveillance system should be implemented.  
4. 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.  
5. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use should only be done in designated areas and are not permitted in animal or procedure rooms.
6. All procedures are carefully performed to minimize the creation of aerosols or splatters.
7. Equipment and work surfaces in the room are routinely decontaminated with an effective disinfectant after work with the infectious agent, and especially after overt spills, splashes, or other contamination by infectious materials.
8. All infectious samples are collected, labeled, transported, and processed in a manner that contains and prevents transmission of the agent(s). All wastes from the animal room (including animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse) are transported from the animal room in leak-proof, covered containers for appropriate disposal in compliance with applicable institutional or local requirements. The outer surface of the containers is disinfected prior to moving the material. Autoclaving of the contents prior to incineration is recommended.
9. Policies for the safe handling of sharps are instituted:
   a. Needles and syringes or other sharp instruments are restricted for use in the animal facility only when there is no alternative, such as for parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
   b. Syringes that re-sheathe the needle, needle-less systems, and other safe devices should be used when appropriate.
   c. Plasticware should be substituted for glassware whenever possible.
10. Personnel wash their hands after handling cultures and animals, after removing gloves, and before leaving the animal facility.
11. A biohazard sign must be posted on the entrance to the animal room whenever infectious agents are present. The hazard warning sign identifies the infectious agent(s) in use, lists the name and telephone number of the responsible person(s), and indicates the special requirements (e.g., the need for immunizations and respirators) for entering the animal room.
12. An insect and rodent control program is in effect (see Appendix G).

B. Special Practices
1. Animal care laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates, or additional training as necessary for procedural or policy changes. Records of all training provided are maintained. In general, persons who may be at increased risk of acquiring infection, or for whom infection might be unusually hazardous, are not allowed in the animal facility unless special procedures can eliminate the extra risk.
2. Only animals used for the experiment(s) are allowed in the room.
3. All equipment must be appropriately decontaminated prior to removal from the room.
4. Spills and accidents which result in overt exposures to infectious materials must be immediately reported to the facility director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

C. Safety Equipment (Primary Barriers)
1. Gowns, uniforms, or laboratory coats are worn while in the animal room. The laboratory coat is removed and left in the animal room. Gowns, uniforms, and laboratory coats are removed before leaving the animal facility. Gloves are worn when handling infected animals and when skin contact with infectious materials is unavoidable.
2. Personal protective equipment is used based on risk assessment determinations (see Section V ). Appropriate face/eye and respiratory protection is worn by all personnel entering animal rooms that house nonhuman primates.
3. Biological safety cabinets, other physical containment devices, and/or personal protective equipment (e.g., respirators, face shields) are used whenever conducting procedures with a high potential for creating aerosols. These include necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, or intranasal inoculation of animals.
4. When needed, animals are housed in primary biosafety containment equipment appropriate for the
animal species. Filter top cages are always handled in properly designed and operating animal biocontainment cabinets recommended for rodents.

D. Facilities (Secondary Barriers)
1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building.
2. Access to the facility is limited by secure locked doors. External doors are self-closing and self-locking. Doors to animal rooms open inward, are self-closing, and are kept closed when experimental animals are present. Cubicle room inner doors may open outward or be horizontal or vertical sliding.
3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors, and ceilings) are water resistant.
4. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas.
5. Any 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.
6. If floor drains are provided, the traps are always filled with an appropriate disinfectant.
7. Exhaust air is discharged to the outside without being recirculated to other rooms. Ventilation should be provided in accordance with criteria from Guide for Care and Use of Laboratory Animals, latest edition. The direction of airflow in the animal facility is inward; animal rooms should maintain negative pressure compared to adjoining hallways.
8. Cages are washed manually or in an appropriate cage washer. The mechanical cage washer should have a final rinse temperature of at least 180F.
9. An autoclave is available in the animal facility to decontaminate infectious waste.
10. A hand washing sink is in the animal room where infected animals are housed, as well as elsewhere in the facility.
11. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.