

## **Biological Safety Manual**

**Summary/Purpose:** The Biological Safety Manual details the minimum requirements and procedures for operations involving Biohazardous Materials.

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### ***Introduction***

The purpose of this manual is to set biological safety policies for the University of Mississippi, Oxford Campus. This manual contains information, regulations and procedures to provide for the safety of personnel and the environment from biologically hazardous materials. It is not intended that this manual be an exhaustive treatment of biological safety and certain work areas will have problems not covered herein. In general, federal and state guidelines dealing with biological safety are not restated herein, but all such guidelines are assumed to be applicable and binding on all personnel working at the Oxford campus of the University.

Information on biological safety is available from the Laboratory Services (LS) for the development of specific safety programs for operations not covered in this manual. Additional information on topics in this manual as well as slide and videocassette seminars are also available from the LS.

### **DEFINITION OF A BIOHAZARD**

Biohazardous agent is one that is biological in nature, capable of self-replication and has the capacity to produce deleterious effects upon other biological organisms, particularly humans.

### **DANGEROUS CHARACTERISTIC**

It is important to distinguish a dangerous characteristic of hazardous biological agents. They all have the ability to replicate. In contrast to chemical agents, infectious biological agents can reproduce which gives rise to the potential of large populations in nature when small numbers are released from a controlled situation. Although SAFE doses of chemical and physical agents are often specified, there is NO SAFE level of a non-contained pathogenic organism.

### **TRAINING IN BIOHAZARDS**

Containment and safe handling of known and potentially biohazardous materials require strict adherence to prudent microbiological practices. Therefore, all personnel directly or indirectly involved with any such materials must receive adequate instruction in these practices. This should include training in appropriate aseptic technique and instruction in the biology of the organism so that the potential biohazards can be understood and appreciated.

Safety in your work involving biohazards depends upon how you or a member of a team applies available guidelines. In addition to specific safety knowledge, motivation and critical judgement are absolutely essential for ensuring the protection of all personnel, the public and the environment.

### **ROUTES OF EXPOSURE**

Exposure and subsequent infection of an individual with a biohazardous agent can occur by several routes. The most common routes are the following:

1. Oral
2. Respiratory
3. Skin puncture
4. Penetration through unbroken skin
5. Conjunctival

All the above routes account for a percentage of known infections, but respiratory exposure probably accounts for 65 to 75% of all infections.

Most routine bacteriological and virological procedures are capable of producing aerosols. In order to initiate a respiratory infection, aerosols must be deposited and retained in the respiratory tract. Particles of less than 5 micrometers are most effective in establishing airborne infections in laboratory animals. Airborne organisms can remain suspended in air for long periods of time and can be carried around the room by convective air currents and also throughout the ventilation systems of buildings. Since these organisms remain infectious for long periods of time, there is a potential danger to anyone who comes in contact with the organisms. In most cases, however, the doses to which an individual may be exposed are insufficient to cause disease. Infectious doses for humans vary with the agent, the route of exposure and the resistance of the host.

***Institutional Biosafety Committee (IBC)***

The institution shall establish an IBC whose responsibilities need not be restricted to recombinant DNA and shall meet the requirements as outlined in Section IV-B-2 of the NIH Guidelines for Research Involving Recombinant DNA Molecules.

- A. The Institutional Biosafety Committee or IBC means a committee that meets the requirements for membership specified by the NIH and reviews, approves, and oversees projects in accordance with the responsibilities defined by the NIH. The IBC shall be established by the institution and represent a collective expertise and experience in recombinant DNA research experiments and potential risk to public health or the environment.
- B. On behalf of the institution, the IBC is responsible for reviewing for compliance with the NIH guidelines for research with pathogens and recombinant DNA research. This review shall include an independent assessment of the containment levels required by these Guidelines for the proposed research, an assessment of the facilities, procedures, and practices, and of the training and expertise of personnel. The IBC will notify the PI and LS of the results of its review. The IBC will also be responsible for setting containment levels as specified by NIH guidelines and direct LS to periodically review laboratory conditions where recombinant DNA or other biohazardous research is being conducted to ensure that the requirements of the Guidelines are being fulfilled.
- C. The IBC will report to the LS institutional official and to the Office of Recombinant DNA Activities at the NIH any significant problems with or violations of the Guidelines and any significant research-related accidents or illness unless the IBC determines that the PI has already done so.

D. The IBC may not authorize initiation of experiments not explicitly covered by the Guidelines until NIH (with the advice of the Recombinant DNA Advisory Committee (NIH) when required) establishes the containment requirement.

E. Performing such other functions as may be delegated to the IBC under personnel descriptions.

### ***Roles and Responsibilities***

Safety in activities involving biohazardous agents depends on the individual conducting them. The Guidelines cannot anticipate every possible situation. Motivation and good judgement are the key essentials to protection of health and the environment. These guidelines are intended to help the institution, Institutional Biosafety Committee (IBC), Laboratory Services (LS) and Principal Investigator (PI) determine the safeguards that should be implemented. These guidelines will never be complete or final in that all conceivable experiments involving recombinant DNA and other biohazardous agents cannot be foreseen, therefore it is the responsibility of the institution and those associated with it to adhere to the intent of the guidelines as well as to their specifics.

Each institution (and the IBC acting on its behalf) is responsible for ensuring that recombinant DNA activities comply with the guidelines. General recognition of institutional authority and responsibility properly establishes accountability for safe conduct of the research at the local level.

### ***Biological Laboratory Safety***

It has been shown that the most common exposure route for pathogenic or disease inducing agents is through the production of aerosols. Any operation that mechanically disturbs a solution has the potential to release a cloud of contaminated droplets into the air. Many of these droplets will be too small to be seen and capable of being transported from the point of origin by air currents. The aerosols may also settle on equipment or hands and be transmitted by subsequent contact. Good technique, therefore, is to minimize production of aerosols. Work with pathogenic or disease inducing agents must be done in containment hoods such as biological hoods at a minimum of BL2 procedures (actual biological safety level is determined by the organism or pathogen).

Basic safety rules for aerosols are as follows:

1. Do not expel the last drop from a pipette. The expulsion creates droplets.
2. Do not mix solutions by pipette.
3. Sterilization of loops or needles by flame causes spattering. The instruments should be cleaned in a decontaminating solution before heating. A shield to catch flying particles should be used.
4. Allow needles and loops to cool before applying them to specimens or cultures.
5. Do not rely on the medium to cool the instruments.
6. Do not streak agar that has a rough or broken surface.
7. Agitate broth cultures only by swirling smoothly in a horizontal plane, not in a vertical fashion.
8. Surround the needle with a disinfectant-soaked cloth while injecting biologically active materials.

9. Wrap the syringe with a disinfectant-soaked cloth when breaking the needle off after use, prior to disposal.
10. Blenders and centrifuges create large quantities of aerosols.

Allow the aerosols to settle for several minutes before opening these devices.

All of the techniques above can only reduce the generation of aerosols, not eliminate them. Where the possibility of infection is significant, the work must be done in a biological safety cabinet or, if the risk is very high or the disease is a serious one, the work must be performed in a glove box. The specifications of these cabinets are given in the section "Biological Safety Cabinets."

In addition to the aerosol control practices covered in the previous section, there are a number of other procedures that must be followed in microbiological or biomedical laboratories. The items listed below are minimum standards that must be followed in every active laboratory. Where the risk is high the investigator will be asked to make a formal presentation of additional safety precautions to the Institutional Biosafety Committee for approval.

1. Laboratory doors should be kept closed when experiments are in progress.
2. Work surfaces are to be decontaminated at least once a day and after each spill or viable material.
3. All contaminated liquid or solid wastes are decontaminated before disposal.
4. Mechanical pipetting devices must be used; mouth pipetting is prohibited.
5. Eating drinking, smoking, storing food, and applying cosmetics are not to be permitted in the work area.
6. Persons must wash their hands when they leave the laboratory.
7. All laboratory operations must conform to the requirements of the Biosafety Level assigned by the Institutional Biosafety Committee, see Appendix C
8. All animal operations must conform to the requirements of the Animal Biosafety Level assigned by the Institutional Animal Care and Use Committee, see Appendix D.

### ***Disposal of Biological Material***

In general, it is the responsibility of the principal investigator or supervisor of a laboratory handling biologically hazardous material to insure its PROPER STERILIZATION prior to disposal either as a chemical hazard, a radiological hazard or as ordinary trash. **NO BIOLOGICAL MATERIAL STORED IN A BIOHAZARDOUS BAG WILL BE DISPOSED OF IN THE ORDINARY UNIVERSITY TRASH.** The biohazardous material must be rendered inactive by sterilization either by the principal. If sterilized by the principal investigator, the material must be removed from the Biohazard Bag prior to disposal in ordinary University trash by the principal investigator.

Autoclaving and dry heat sterilization will be the responsibility of the principal investigator or laboratory supervisor.

### **DISPOSAL OF MEDICAL WASTE**

Laboratory Services (LS) is responsible for compliance with and enforcement of the federal and state laws and regulations that apply to the use and disposal of biologically hazardous materials. Specific regulations and policies are located in the [Biological Safety Manual](#)

Federal regulations currently divide biological waste into two categories - **Medical Waste** and **Pathological Waste** The simplified definition is that biological waste produced in research and in the testing of materials is Medical and biological waste produced in classrooms or in educational settings are Pathological.

**Medical Waste = Research / Testing - - - - - Pathological Waste = Classroom / Education**

Medical Waste disposal requires the services of an outside contractor and the segregation of waste materials at the departmental level.

**Follow the packaging guidelines from the vendor – published on the LS Web site**

### **DRY HEAT STERILIZATION**

For the sterilization of sharps, syringes, broken glass, etc. which are contaminated with a biohazard, it is recommended that dry heat be used, e.g., a laboratory drying oven that is capable of temperatures up to 200 ° C. Laboratory Services does not have the facilities for this type of sterilization. As a result, it is the responsibility of the individual investigator or his or her department to provide this type of sterilization, if it is needed.

A. The following table gives the recommended temperature and time for various biohazards:

#### **DRY HEAT TEMPERATURE/TIME CYCLE**

<b>--- BIO-HAZARD - - - - -</b>	<b>TEMPERATURE - - - - -</b>	<b>TIME</b>
--- Non-sporing organisms	--- 100 ° C- - - - -	--- 1 hr.
--- Fungal spores	--- 120 ° C- - - - -	--- 1 hr.
--- Bacterial spores	--- 160 ° C- - - - -	--- 1 hr.
--- Resistant spores	--- 180 ° C- - - - -	--- 1 hr.
--- Resistant viruses	--- 200 ° C- - - - -	--- 1 hr.

### **STEAM AUTOCLAVES**

The recommended method for sterilization of non-burnable biohazardous material is steam autoclaving. Since treatment conditions to achieve sterility will vary in relation to the volume of materials treated, its contamination level, the moisture content, and other factors care should be

exercised in using this method of sterilization. In general, it is recommended that the autoclave be operated at a temperature of 121° C and run for a minimum of one hour.

However, the recommendations of the manufacturer of the particular autoclave should be followed in order to provide an adequate pressure/temperature/time cycle to ensure sterilization. It is also recommended that plastic autoclaving bags with a color indicator be used to give a visual indication that the material is indeed sterile. This is especially important when highly potent biological agents are involved.

The use of sterilization tape is required on all contaminated materials undergoing all types of sterilizations listed above.

LS will test all autoclaves on the Oxford campus twice a year, usually in January and July, for proper operation.

## **LIQUID DISINFECTION**

An alternative method to dry heat sterilization of sharps, glass, etc. is the disinfection of the material using a chemical agent. The recommended method is to soak the sharps, glass, and other materials in full strength Chlorox for a period of one hour. Alternative disinfecting agents are: 70-85% ethanol, 5% formaldehyde solution, and Wescodyne diluted to give 75 ppm of free iodine.

### ***Shipment of Biological Agents***

#### **Refer to the current LS Shipping Policies and regulations**

#### ***Recombinant DNA***

1. DNA molecules which are constructed outside living cells by joining foreign\* natural or foreign\* synthetic DNA segments to DNA molecules that can replicate in a living cell;
2. DNA molecules that result from the replication of those described in (1). Synthetic DNA segments likely to yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent) will be considered as equivalent to their natural DNA counterparts. If the synthetic DNA segment is not expressed "in vivo" as a biologically active polynucleotide or polypeptide product, it is exempt from the Guidelines.

\*(Rearrangements involving the introduction of DNA from different organisms or different strains of an organism will be considered recombinant DNA. Deletions, single-base changes and rearrangements within a single genome will not involve the introduction of foreign DNA and therefore would not be considered recombinant DNA.)

## **GENERAL APPLICABILITY**

The recombinant DNA guidelines are applicable to all recombinant DNA research within the United States or its territories which is conducted at or sponsored by an institution that receives

any support for recombinant DNA research from the National Institutes of Health. Any individual receiving support for research involving recombinant DNA must be associated with or sponsored by an institution that can and does assume the responsibilities assigned in these guidelines.

## **CONTAINMENT**

Effective biological safety programs have been operative in a variety of laboratories for many years. Considerable information exists in the design of physical containment facilities and the selection of laboratory procedures applicable to organisms carrying recombinant DNAs. The existing programs can be divided into two categories: (i) A set of standard practices that are generally used in microbiological laboratories; and (ii) special procedures, equipment, and laboratory installations that provide physical barriers which are applied in varying degrees according to the estimated biohazard. Four biosafety levels consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities appropriate for the operations performed and the hazards posed by agents and for the laboratory function and activity. Biosafety level 4 (BL4) provides the most stringent containment conditions, BL1 the least stringent.

Experiments on recombinant DNAs by their nature lend themselves to a third containment mechanism (iii), namely, the application of highly specific biological barriers. In fact, natural barriers do exist which limit either (1) the infectivity of a "vector" or "vehicle" (plasmid or virus) for specific hosts, or (2) its dissemination and survival in the environment. The vectors that provide the means for replication of the recombinant DNAs and/or the host cells in which they replicate can be genetically designed to decrease by many orders of magnitude the probability of dissemination for recombinant DNAs outside the laboratory.

## **GUIDELINES FOR COVERED EXPERIMENTS**

Experiments involving recombinant DNA can be divided into four general classes as described below.

- A. Experiments which require specific RAC (Recombinant DNA Advisory Committee) review and NIH and IBC approval before initiation of the experiment.
- B. Experiments which require IBC approval before initiation of the experiment.
- C. Experiments which require IBC notification at the time of initiation of the experiment.
- D. Experiments which are exempt from the procedures of the **GUIDELINES**.

**IF AN EXPERIMENT FALLS INTO BOTH CLASS "A" AND ONE OF THE OTHER CLASSES, THE RULES PERTAINING TO CLASS "A" MUST BE FOLLOWED!**



E. "Class A" Experiments - (require specific RAC (Recombinant DNA Advisory Committee) review and NIH and IBC approval before initiation of the experiment).

Experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH, the publication of the Federal Register for thirty days of comment, review by the RAC, and specific approval by NIH. Included in this guideline (taken from the Federal Register, Vol. 51, No. 88, Wed. May 7, 1986, 16960) are the following criteria.

1. Deliberate formation of recombinant DNAs containing genes for the biosynthesis of toxic molecules lethal for vertebrates at an LD50 of less than 100 ng per kg body weight (e.g., microbial toxins such as botulinum toxins, tetanus toxin, diphtheria toxin, Shigella dysenteriae neurotoxin).
2. Deliberate release into the environment of any organism containing DNA.
3. Deliberate transfer of a drug resistant trait to microorganisms that are not known to acquire it naturally if such acquisition could compromise the use of the drug to control disease agents in human or veterinary medicine or agriculture.
4. Deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into human subjects.

**F. Class B Experiments - Experiments which require IBC approval before initiation of the experiment).**

Investigators performing experiments in this category must submit to their IBC, prior to initiation of the experiments, a registration document that contains a description of the source(s) of DNA, the nature of the inserted DNA sequences, the hosts and vectors to be used, whether a deliberate attempt will be made to obtain expression of a foreign gene, and if so what protein will be produced, and the containment conditions specified in this Guideline. Registration document must be dated and signed by the investigator and filed only with the local IBC. The IBC shall review all such proposals prior to initiation of the experiments. Included in this guideline (taken from the Federal Register, Vol. 51, No. 88, Wed. May 7, 1986, 16960) are the following criteria.

1. Experiments using human or animal pathogens (Class 2, 3, 4, or 5 agents) as host vectors.
2. Experiments in which DNA from human or animal pathogens (Class 2, 3, 4, or 5) agents is cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector systems.
3. Experiments involving the use of infectious animal or plant DNA or RNA viruses or defective animal or plant DNA or RNA viruses in the presence of helper virus in tissue culture systems.
4. Recombinant DNA experiments involving whole animals or plants.

5. Experiments involving more than 10 liters of culture. (Physical containment for large-scale uses of organisms containing recombinant DNA molecules should be used, see appendix K for guidelines).

G. Class C Experiments - Experiments which require IBC notification at the time of initiation of the experiment.

Experiments not included in "A or B" categories are to be considered as Class C experiments. All such experiments can be carried out at BL1 containment. For experiments in this category, a registration document as described in for Class B experiments must be dated and signed by the investigator and filed with the local IBC at the time of initiation of the experiment. The IBC shall review all such proposals, but IBC review prior to initiation of the experiment is not required. For example, experiments in which all components derive from non-pathogenic lower eukaryotes fall under Class C experiments and can be carried out at BL1 containment.

H. Class D Experiments - Exempt from Guidelines.

The following recombinant DNA molecules are exempt from the guidelines for research involving recombinant DNA molecules and no registration with the IBC is necessary.

1. Those that are not in organisms or viruses.
2. Those that consist entirely of DNA segments from a single nonchromosomal or viral DNA source though one or more of the segments may be a synthetic equivalent.
3. Those that consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species) or when transferred to another host by well established physiological means; also, those that consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).
4. Certain specified recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the Director, NIH, with the advice of the RAC (Recombinant DNA Advisory Committee) after appropriate notice and opportunity for public comment.
5. Other classes of recombinant DNA molecules - if the Director, NIH, with advice of the RAC, after appropriate notice and opportunity for public comment, finds that they do not present a significant risk to health or the environment.

### ***Regulations for Working with Biohazardous Organisms***

## **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. Primary containment, the protection of personnel and the immediate laboratory environment from exposure to infectious agents, is provided by good microbiological technique and the use of appropriate safety equipment. The use of vaccines may provide an increased level of personal protection. Secondary containment, the protection of the environment external to the laboratory from exposure to infectious materials, is provided by a combination of facility design and operational practices. The purpose of containment is to reduce exposure of laboratory workers and other persons to, and to prevent escape into the outside environment of potentially hazardous agents. The three elements of containment include laboratory practice and technique, safety equipment, and facility design.

## **PHYSICAL CONTAINMENT**

The objective of physical containment is to confine pathogenic and organisms containing recombinant DNA molecules and thus to reduce the potential for exposure of the laboratory worker, persons outside of the laboratory, and the environment to such organisms. Physical containment is achieved through the use of laboratory practices, containment equipment, and special laboratory design.

Emphasis is placed on primary means of physical containment which are provided by laboratory practices and containment equipment. Special laboratory design provides a secondary means of protection against the accidental release of organisms outside the laboratory or to the environment. Special laboratory design is used primarily in facilities in which experiments of moderate to high potential hazards are performed.

Combinations of laboratory practices, containment equipment, and special laboratory design can be made to achieve different levels of physical containment. Four levels of physical containment, which are designated as BL1, BL2, BL3, and BL4, are described. It should be emphasized that the descriptions and assignments of physical containment detailed below are based on existing approaches to containment of pathogenic organisms. The National Cancer Institute describes three levels for research on oncogenic viruses which roughly correspond to the BL2, BL3, and BL4 levels.

It is recognized that several different combinations of laboratory practices, containment equipment, and special laboratory design may be appropriate for containment of specific research activities. The NIH Guidelines for Research Involving Recombinant DNA Molecules, therefore allow alternative selections of primary containment equipment within facilities that have been designed to provide BL3 and BL4 levels of physical containment. The selection of alternative methods of primary containment is dependent on the level of biological containment provided by the host-vector system used in the experiment.

## **LABORATORY PRACTICE AND TECHNIQUE**

The most important element of containment is strict adherence to standard microbiological 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 safely handling such material. Included in this training are aseptic techniques and in the biology of the organism used in the experiments so that the potential biohazards can be understood and appreciated. The principal investigator is responsible for ensuring appropriate training of personnel who will be involved with the project.

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 laboratory director is responsible for selecting additional safety practices, which must be in keeping with the hazard associated with the particular agent(s) or procedure(s).

Each laboratory should develop or adopt a biosafety or operations manual which identifies the hazards that will or may be encountered and which specifies practices and procedures designed to minimize or eliminate risks. 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 handling infectious agents must direct laboratory activities.

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

Any research group working with agents with a known or potential biohazard shall have an emergency plan which describes the procedures to be followed if an accident contaminates personnel or the environment for each potential biohazard used in the project. The PI must insure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan. If a research group is working with a known pathogen for which there is an effective vaccine, the vaccine should be made available to all workers.

### **SAFETY EQUIPMENT (PRIMARY BARRIERS)**

Safety equipment includes biological safety cabinets and a variety of enclosed containers. The biological safety cabinet is the principal device used to provide containment of infectious aerosols generated by many microbiological procedures. Three types of biological safety cabinets (Class I, II, III) used in microbiological laboratories are illustrated in Figures 1-3 and described in Appendix A. Open fronted Class I and Class II biological safety cabinets are partial containment cabinets which offer significant levels of protection to laboratory personnel and to the environment when used with good microbiological techniques. The gas-tight Class III biological safety cabinet provides the highest attainable level of protection to personnel and to the environment. An example of an enclosed container is the safety centrifuge cup, which is designed to prevent aerosols from being released during centrifugation.

Safety equipment also includes items for personal protection such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, and safety glasses. These personal protective devices are often used in combination with biological safety cabinets and other devices which contain the agents, animals or materials being worked with. In some situations in which it is impractical to work in biological safety cabinets, personal protective devices may form the primary barrier

between personnel and the infectious materials. Examples of such activities include certain animal studies, animal necropsy, production activities, and activities relating to maintenance service, or support of the laboratory facility.

***Regulations for Working with Human Blood, Fluids of Tissue***

Before research involving human blood, blood products, or human pathogens can be undertaken, the following requirements must be met prior to the start of the project:

1. The principal investigator must register the project with the LS.
2. A written and detailed protocol for the project work must be submitted to LS for review and approval by the Institutional Biosafety Committee. The IBC will assign the Biosafety Level for the laboratories involved in the project.
3. The personnel involved in the project must receive safety training proper to the specific project as determined by the IBC.
4. The facilities in which the project is to be carried out must be inspected by LS to determine if they meet the Biosafety level assigned to the project.
5. The safety equipment required by LS for the project must be provided by either the grant or the department prior to the start of the project.
6. The method of biohazardous waste disposal for the specific project must be approved by LS.
7. Work on each specific project will not start until all safety requirements determined by LS have been met and the project is formally approved by LS and the IBC. Written notice of approval will be provided on form LS-98 for Blood and Blood Products or on form LS-99 for Human Pathogens.

***Guidelines for Handling Human Blood and Blood Products***

Many programs in the University require the handling a variety of potentially infectious materials. This is especially true of the health care clinics, clinical laboratories, and several of the laboratory science programs. Microbiological cultures, urine samples, blood and blood products, and the materials and equipment which come into contact with these substances are among the sources of biologically hazardous agents in use in typical on-campus research laboratories and many laboratory courses. These materials have the ability to transmit pathogenic bacteria and blood borne viruses such as human immunodeficiency virus (HIV) and hepatitis B.

Those who are in contact with the biologically hazardous materials used in the classroom must be protected. These include faculty, students, laboratory assistants, housekeeping staff members, and refuse handlers.

## The University of Mississippi

The need to label, correctly store, and properly dispose of hazardous chemicals in the teaching laboratory has become widely recognized. Work place safety regulations were once not readily applied to college and university classrooms. However, these regulations are now understood to apply in the classroom; and state and federal inspectors are beginning to make visits to education work sites to ensure that chemicals are handled safely and disposed of properly. So, too, it must be recognized that regulations regarding the handling and disposal of infectious materials apply to education settings where these materials are used just as they apply to the health care providers. Federal regulations of hazardous materials in the work place which apply to clinical laboratory education programs are as follows:

1. **Gloves:** Disposable gloves are required when performing laboratory tests on blood and body fluids or when handling bagged contaminated materials. The regulation does not require wearing gloves if venipuncture is performed by students in the teaching setting unless hands are abraded or have active dermatitis. However, CDC guidelines recommend that persons who are just learning the techniques of venipuncture should wear gloves.
2. **Masks:** Masks for eyes and mouth are required when these parts of the body may be exposed to infectious materials. The most common times when aerosols or spills of blood occur in the clinical laboratory science course are when rubber stoppers are removed from tubes and during centrifugation. Sprays and splashes of contaminated fluids may also occur if students are required to perform maintenance on machines with hydraulic lines.
3. **Laboratory coats:** Laboratory coats or gowns, as well as gloves, must be worn when students perform tests on laboratory specimens.
4. **Labeling infectious wastes:** Contaminated materials and infectious waste materials must be stored in leak proof containers with the word "Biohazard" or the biological hazard symbol displayed in a manner which is readable five feet away. Single or double plastic bags which are at least 3 mils thick are commonly used. Orange bags are used for materials which remain infectious when they leave the classroom laboratory.
5. **Needle disposal:** Needles must not be recapped, bent, or broken. They must be placed in a dedicated container which is labeled, closable, puncture resistant, and splatter proof.
6. **Training:** Training in the proper techniques and types of infectious hazards must be provided for instructors and other laboratory personnel who handle infectious material. Records must be kept which show that this training has taken place.
7. **Cleaning of spills:** A 10% chlorine bleach solution is recommended to disinfect spilled infectious material. In addition, the CDC recommends that this type of disinfecting solution should be used to clean surfaces and instruments which are knowingly or possibly inadvertently contaminated with infectious material.
8. **Storage of infectious waste:** Infectious waste can be stored at room temperature for no more than 72 hrs.

The two acceptable methods for rendering contaminated solid materials noninfectious are steam autoclaving and incineration. These two processes require that a steam sterilizer must be operated at 250°F for 90 min at 15 psi pressure and that an incinerator must have a burn temperature of not less than 1400°F and the gases generated must be subjected to a temperature of not less than 1800°F.

Most clinical laboratory science programs have access to an autoclave and find this to be the most economical method of dealing with infectious materials and contaminated solid wastes. Autoclave tape should be placed on the bag prior to sterilization to indicate that it has been decontaminated. Following steam sterilization, the waste may be placed in the general refuse. Not all institutions have access to incinerators, but incineration services may be provided through commercial waste handlers. The drawbacks to using a commercial service are the expense and the storage time needed between pick-ups. Because education programs generate much less waste than health care providers, they may take several weeks to generate enough waste to justify the cost of a pick-up by a commercial service.

The 1987 CDC "Recommendations for the Prevention of HIV Transmission in Health Care Settings" suggests that some infective wastes can be poured safely down the drain. The recommendations indicate that there is some subjectivity in such a decision. If a substance in a residential setting, such as urine, would normally be disposed of by way of the sewer, a similar procedure may be used in the health care or clinical education setting. However, if there is a question about the proper procedure for disposal, steam sterilization or incineration is recommended.

In the case of known infectious agents, or experiments using human or animal pathogens, the principal investigator is referred to procedures outlined previously in "Guidelines for Research with Recombinant DNA and Other Biohazardous Organisms."

### ***Regulations for Working with Animals***

The use of animals for teaching and research is controlled by the Institutional Animal Care and Use Committee (IACUC) which is administered through the Office of Research.

### **Facility Design**

The design of the facility is important in providing a barrier to protect persons working in the facility but outside the laboratory and those in the community from infectious agents which may be accidentally released from the laboratory. Laboratory management is responsible for providing facilities commensurate with the laboratory's function. Three facility designs are described below, in ascending order by level of containment.

**The Basic Laboratory.** This laboratory provides general space in which work is done with viable agents which are not associated with disease in healthy adults. Basic laboratories include those facilities described in the following pages as Biosafety Levels 1 and 2 facilities. This laboratory is also appropriate for work with infectious agents or potentially infectious materials when the hazard levels are low and laboratory personnel can be adequately protected by standard laboratory practice. While work is commonly conducted on the open bench, certain operations

are confined to biological safety cabinets. Conventional laboratory designs are adequate. Areas known to be sources of general contamination, such as animal rooms and waste staging areas, should not be adjacent to patient care activities. Public areas and general offices to which nonlaboratory staff require frequent access should be separated from spaces which primarily support laboratory functions.

**The Containment Laboratory.** This laboratory has special engineering features which make it possible for laboratory workers to handle hazardous materials without endangering themselves, the community, or the environment. The containment laboratory is described in the following pages as a Biosafety Level 3 facility. The unique features which distinguish this laboratory from the basic laboratory are the provisions for access control and a specialized ventilation system. The containment laboratory may be an entire building or a single module or complex of modules within a building. In all cases, the laboratory is separated by a controlled access zone from areas open to the public.

**The Maximum Containment Laboratory.** This laboratory has special engineering and containment features that allow activities involving infectious agents that are extremely hazardous to the laboratory worker or that may cause serious epidemic disease to be conducted safely. The maximum containment laboratory is described on the following pages as a biosafety Level 4 facility. Although the maximum containment laboratory is generally a separate building, it can be constructed as an isolated area within a building. The laboratory's distinguishing characteristic is that it has secondary barriers to prevent hazardous materials from escaping into the environment. Such barriers include sealed openings into the laboratory, airlocks or liquid disinfectant barriers, a clothing-change and shower room contiguous to the laboratory, a double door autoclave, a biowaste treatment system, a separate ventilation system, and a treatment system to decontaminate exhaust air.

### ***Biosafety Level 1***

Practices, safety equipment, and facilities are appropriate for undergraduate and secondary educational training and teaching laboratories and for other facilities in which work is done with defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans. *Bacillus subtilis*, *Naegleria gruberi*, and infectious canine hepatitis virus are representative of those microorganisms meeting these criteria. Many agents not ordinarily associated with disease processes in humans are, however, opportunistic pathogens and may cause infection in the young, the aged, and in immunodeficient or immunosuppressed individuals.

Biosafety Level 1 is suitable for work involving agents of no known or of minimal potential hazard to laboratory personnel and the environment. The laboratory is not separated from the general traffic patterns in the building. Work is generally conducted on open bench tops. Special containment equipment is not required or generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science. The following standard and special practices, safety equipment, and facilities apply to agents assigned to

Biosafety Level 1:



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### 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. Work surfaces are decontaminated once a day and after any spill of viable material.
3. All contaminated liquid or solid wastes are decontaminated before disposal.
4. Mechanical pipetting devices are used; mouth pipetting is prohibited.
5. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only. Food storage cabinets or refrigerators should be located outside of the work area.
6. Persons wash their hands after they handle viable materials and animals and before leaving the laboratory.
7. All procedures are performed carefully to minimize the creation of aerosols.
8. It is recommended that laboratory coats, gowns, or uniforms be worn to prevent contamination or soiling of street clothes.

### B. Special Practices

1. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leakproof container which is closed before being removed from the laboratory.
2. An insect and rodent control program is in effect.

C. Special containment equipment is generally not required for manipulations of agents assigned to Biosafety Level 1.

### D. Laboratory Facilities

1. The laboratory is designed so that it can be easily cleaned.
2. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
3. Laboratory furniture is sturdy. Spaces between benches, cabinets, and equipment are accessible for cleaning.
4. Each laboratory contains a sink for handwashing.

5. If the laboratory has windows that open, they are fitted with fly screens.

### ***Biosafety Level 2***

Practices, equipment, and facilities are applicable to clinical, diagnostic, teaching and other facilities in which work is done with the broad spectrum of indigenous moderate-risk agents present in the community and associated with human disease of varying severity. With good microbiological techniques, these agents can be used safely in activities conducted on the open bench, provided the potential for producing aerosols is low. Hepatitis B virus, the salmonellae, and *Toxoplasma* spp. are representative of microorganisms assigned to this containment level. Primary hazards to personnel working with these agents may include accidental autoinoculation, ingestion, and skin or mucous membrane exposure to infectious materials. Procedures with high aerosol potential that may increase the risk of exposure of personnel must be conducted in primary containment equipment or devices.

Biosafety Level 2 is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that:

- a) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists,
- b) access to the laboratory is limited when work is being conducted, and,
- c) Certain procedures in which infectious aerosols are created are conducted in biological safety 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 by the laboratory director when work with infectious agents or recombinant DNA molecules is in progress.
2. Work surfaces are decontaminated at least once a day and after any spill of viable material.
3. All infectious liquid or solid wastes are decontaminated before disposal.
4. Mechanical pipetting devices are used; mouth pipetting is prohibited.
5. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only. Food storage cabinets or refrigerators should be located outside of the work area.
6. Persons wash their hands after handling infectious materials or organisms containing recombinant DNA molecules, animals, and when they leave the laboratory.
7. All procedures are performed carefully to minimize the creation of aerosols.

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8. Experiments of lesser biohazard potential can be carried out concurrently in carefully demarcated areas of the same laboratory.

### B. Special Practices

1. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leakproof container which is closed before being removed from the laboratory.

2. The laboratory director limits access to the laboratory. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

3. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific entry requirements (e.g., immunization) enter the laboratory and animal rooms.

4. When the infectious agent(s) or organisms containing recombinant DNA molecules are in use in the laboratory require special provisions for entry (e.g., vaccination), a hazard warning sign, incorporating the universal biohazard symbol, is posted on the access door to the laboratory work area. The hazard warning sign identifies the infectious agent, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates the special requirement(s) for entering the laboratory.

5. An insect and rodent control program is in effect.

6. Laboratory coats, gowns, smocks, or uniforms are worn while in the laboratory. Before leaving the laboratory for non-laboratory areas (e.g., cafeteria, library administrative offices), this protective clothing is removed and left in the laboratory or covered with a clean coat not used in the laboratory.

7. Animals not involved in the work being performed are not permitted in the laboratory.

8. Special care is taken to avoid skin contamination with infectious materials or organisms containing recombinant DNA molecules; gloves should be worn when handling experimental animals and when skin contact with the agent is unavoidable.

9. All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

10. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organism that contain recombinant DNA molecules or infectious agents. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the sheath or guard or removed from the syringe following use. The

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needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

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

12. 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.

13. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read instructions on practices and procedures and to follow them.

### C. Containment Equipment

Biological safety cabinets (Class I or II) (see Appendix A) or other appropriate personal protective or physical containment devices are used whenever:

1. Procedures with a high potential for creating infectious aerosols 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 eggs.
2. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed heads or centrifuge safety caps are used and if they are opened only in a biological safety cabinet.

### D. Laboratory Facilities

1. The laboratory is designed so that it can be easily cleaned.
2. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
3. Laboratory furniture is sturdy, and spaces between benches, cabinets, and equipment are accessible for cleaning.
4. Each laboratory contains a sink for handwashing.
5. If the laboratory has windows that open, they are fitted with fly screens.
6. An autoclave for decontaminating infectious laboratory wastes is available.

### ***Biosafety Level 3***

Practices, safety equipment, and facilities are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents where the potential for infection by aerosols is real and the disease may have serious or lethal consequences. Autoinoculation and ingestion also represent primary hazards to personnel working with these agents. Examples of such agents for which Biosafety Level 3 safeguards are generally recommended include *Mycobacterium tuberculosis*, St. Louis encephalitis virus, and *Coxiella burnetii*.

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents and are supervised by competent scientists who are experienced in working with these agents. All procedures involving the manipulation of infectious material are conducted within biological safety cabinets or other physical containment devices or by personnel wearing appropriate personal protective clothing and devices. The laboratory has special engineering and design features. It is recognized, however, that many existing facilities may not have all the facility safe-guards recommended for Biosafety Level 3 (e.g., access zone, sealed penetrations, and directional airflow, etc.). In these circumstances, acceptable safety may be achieved for routine or repetitive operations (e.g., diagnostic procedures involving the propagation of an agent for identification, typing, and susceptibility testing) in laboratories where facility features satisfy Biosafety Level 2 recommendations provided the recommended "Standard Microbiological Practices," "Special Practices," and "Containment Equipment" for Biosafety Level 3 are rigorously followed. The decision to implement this modification of Biosafety Level 3 recommendations should be made only by the laboratory director.

The following standard and special safety practices, equipment and facilities apply to agents assigned to Biosafety Level 3.

#### A. Standard Microbiological Practices

1. Work surfaces are decontaminated at least once a day and after any spill of viable material.
2. All infectious liquid or solid wastes are decontaminated before disposal.
3. Mechanical pipetting devices are used; mouth pipetting is prohibited.
4. Eating, drinking, smoking, storing food and applying cosmetics are not permitted in the work area.
5. Persons wash their hands after handling infectious materials, animals, organisms that contain recombinant DNA molecules and when they leave the laboratory.
6. All procedures are performed carefully to minimize the creation of aerosols.

7. Persons under 16 years of age shall not enter the laboratory.

8. If experiments involving other organisms which require lower levels of containment are to be conducted in the same laboratory concurrently with experiments requiring BL3 level physical containment, they shall be conducted in accordance with all BL3 level laboratory practices.

#### B. Special Practices

1. Laboratory doors are kept closed when experiments are in progress.

2. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak proof container which is closed before being removed from the laboratory.

3. The laboratory director controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

4. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures enter the laboratory or animal rooms.

5. When infectious materials or organisms containing recombinant DNA molecules or experimental animals are present in the laboratory or containment module, a hazard warning sign, incorporating the universal biohazard symbol, is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates any special requirements for entering the laboratory, such as the need for immunizations, respirators, or other personal protective measures.

6. All activities involving infectious materials or organisms containing recombinant DNA molecules are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench.

7. The work surfaces of biological safety cabinets and other containment equipment are decontaminated when work with infectious materials or organisms containing recombinant DNA molecules is finished. Plastic-backed paper toweling used on non-perforated work surfaces within biological safety cabinets facilitates clean-up.

8. An insect and rodent control program is in effect.

9. Laboratory clothing that protects street clothing (e.g., solid front or wrap-around gowns, scrub suits, coveralls) is worn in the laboratory. Laboratory clothing is not worn outside the laboratory, and it is decontaminated before being laundered.

10. Special care is taken to avoid skin contamination with infectious materials or organisms containing recombinant DNA molecules; gloves should be worn when handling infected animals and when skin contact with infectious materials is unavoidable.
11. Molded surgical masks or respirators are worn in rooms containing infected animals.
12. Animals and plants not related to the work being conducted are not permitted in the laboratory.
13. Laboratory animals held in a BL# area shall be housed in partial-containment caging systems such as Horsfall units, open cages placed in ventilated enclosures, solid-wall and bottom cages covered by filter bonnets on holding racks equipped with ultraviolet in radiation lamps and reflectors. Note: Conventional caging systems may be used provided that all personnel wear appropriate personal protective devices. These shall include at a minimum wraparound gowns, head covers, gloves, shoe covers, and respirators. All personnel shall shower on exit from areas where these devices are required.
14. All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.
15. Vacuum lines are protected with high efficiency particulate air (HEPA) filters and liquid disinfectant traps.
16. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the infection or aspiration of infectious fluids or organisms containing recombinant DNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the sheath or guard or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.
17. Spills and accidents which result in overt or potential exposures to infectious materials or organisms containing recombinant DNA molecules are immediately reported to the laboratory director. Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained.
18. Baseline serum samples for all laboratory and other at-risk personnel should be collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the laboratory.
19. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read instructions on practices and procedures and to follow them.

20. Alternate Selection of Containment Equipment- Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified can be conducted in a BL3 laboratory using containment equipment specified for the BL2 level of physical containment. Experimental procedures involving a host-vector system that provides a one-step lower level of biological containment than that specified can be conducted in a BL3 laboratory using containment equipment specified for the BL4 level of physical containment.

#### C. Containment Equipment

Biological safety cabinets (Class I, II, or III) or other appropriate combinations of personal protective or physical containment devices (e.g., special protective clothing, masks, gloves, respirators, centrifuge safety cups, sealed centrifuge rotors, and containment caging for animals) are used for all activities with organisms containing recombinant DNA molecules which pose a threat of aerosol exposure. These include manipulation of cultures and of those clinical or environmental materials which may be a source of aerosols; the aerosol challenge of experimental animals; and harvesting of infected tissues or fluids from experimental animals and embryonate eggs; and necroscopy of experimental animals.

#### D. Laboratory Facilities

1. The laboratory is separated from areas which are open to unrestricted personnel traffic within the building. Passage through two sets of doors is the basic requirement for entry into the animal room from access corridors or other contiguous areas. Physical separation of the high containment laboratory from access corridors or other activities may also be provided by a double doored clothes change room (showers may be included), airlock, or other access facility which requires passage through two sets of doors before entering the laboratory.
2. The interior surfaces of walls, floors, and ceilings are water resistant so that they may be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate decontaminating the area.
3. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
4. Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.
5. Each laboratory contains a sink for handwashing. The sink is foot, elbow, or automatically operated and is located near the laboratory exit door.
6. Windows in the laboratory are closed and sealed.
7. Access doors to the laboratory or containment module are self-closing.
8. An autoclave for decontaminating laboratory wastes is available, preferably within the laboratory.



9. A duct exhaust air ventilation system is provided. This system creates directional airflow that draws air into the laboratory through the entry area. The building exhaust can be used for this purpose if the exhaust air is not recirculated to any other area of the building, is discharged to the outside, and is dispersed away from occupied areas and air intakes. Personnel must verify that the direction of the airflow (into the laboratory) is proper. The exhaust air from the animal room that does not pass through biological safety cabinets or other primary containment equipment can be discharged to the outside without being filtered or otherwise treated.

8. The HEPA filtered exhaust air from Class I or Class II biological safety cabinets or other primary containment devices is discharged directly to the outside or through the building exhaust system. Exhaust air from these primary containment devices may be recirculated within the laboratory if the cabinet is tested and certified at least every 12 months. If the HEPA filtered exhaust air from Class I or Class II biological safety cabinets is discharged to the outside through the building exhaust system, it is connected to this system in a manner (e.g., thimble unit connection) that avoids any interference with the air balance of the cabinets or building exhaust system.

### ***Animal Biosafety Level 1***

#### **A. Standard Practices**

1. Doors to animal rooms open inward, are self-closing, and are kept closed when experimental animals are present.
2. Work surfaces are decontaminated once a day and after any spill of viable material.
3. Eating, drinking, smoking, and applying cosmetics are not permitted in the animal rooms.
4. Persons wash their hands after they handle viable materials and animals and before leaving the laboratory.
5. All procedures are performed carefully to minimize the creation of aerosols.
6. An insect and rodent control program is in effect.

#### **B. Special Practices**

1. Bedding materials from animal cages are removed in such a manner as to minimize the creation of aerosols and disposed of in compliance with applicable institutional or local requirements.
2. Cages are washed manually or in a cage washer. Temperature of the final rinse water in a mechanical washer should be 180oF.
3. The wearing of laboratory coats, gowns, or uniforms in the animal room is recommended. It is further recommended that laboratory coats worn in the animal room not be worn in other areas.

### C. Containment Equipment

Special containment equipment is not required for animals infected with agents assigned to Biosafety Level 1.

### D. Animal Facilities

1. The animal facility is designed and constructed to facilitate cleaning and housekeeping.
2. A handwashing sink is available in the animal facility.
3. If the animal facility has windows that open, they are fitted with fly screens.
4. It is recommended but not required that the direction of air flow in the animal facility is inward and that exhaust air is discharged to the outside without being recirculated to other rooms.

### ***Animal Biosafety Level 2***

#### A. Standard Practices

1. Doors to animal rooms open inward, are self-closing, and are kept closed when experimental animals are present.
2. Work surfaces are decontaminated once a day and after any spill of viable material.
3. Eating, drinking, smoking, and applying cosmetics are not permitted in the animal rooms.
4. Persons wash their hands after they handle viable materials and animals and before leaving the laboratory.
5. All procedures are performed carefully to minimize the creation of aerosols.
6. An insect and rodent control program is in effect.

#### B. Special Practices

1. Cages are decontaminated, preferably by autoclaving, before they are cleaned and washed.
2. Surgical-type masks are worn by all personnel entering animal rooms housing nonhuman primates.
3. Laboratory coats, gowns, or uniforms are worn while in the animal room. This protective clothing is removed before leaving the animal facility.
4. The laboratory or animal facility director limits access to the animal room to personnel who have been advised of the potential hazard and who need to enter the room for program or service

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purposes when work is in progress. 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 room.

5. The laboratory or animal facility director establishes policies and procedures where only persons who have been advised of the potential hazard and meet any specific requirements (e.g. for immunization) may enter the animal room.

6. When the infectious agent(s) in use in the animal room requires special entry provisions (e.g. vaccination), a hazard warning sign, incorporating the universal biohazard symbol, is posted on the access door to the animal room. The hazard warning sign identifies the infectious agent, lists the name and telephone number of the animal facility supervisor or other responsible persons(s), and indicates the special requirement(s) for entering the animal room.

7. Special care is taken to avoid skin contamination with infectious materials; gloves should be worn when handling infected animals and when skin contact with infectious materials is unavoidable.

8. All wastes from the animal room are appropriately decontaminated-preferably by autoclaving-before disposal. Infected animal carcasses are incinerated off site after being transported from the animal room in leakproof, covered containers.

9. Hypodermic needles and syringes are used only for the parenteral injection or aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable needle syringe units (i.e. the needle is integral to the syringe) are used for the injection or aspiration of infectious fluids. Needles should not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

10. If floor drains are provided, the drain traps are always filled with water or a suitable disinfectant.

11. When appropriate, considering the agents handled, 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.

### C. Containment Equipment

Biological safety cabinets, other physical containment devices, and/or personal protective devices (e.g., respirators, face shields) are used whenever procedures with a high potential for creating aerosols are conducted. These include necroscopy of infected animals, harvesting of infected tissues or fluids from animals or eggs, intranasal inoculation of animals, and manipulation of high concentrations or large volumes of infectious materials.

### D. Animal Facilities

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1. The animal facility is designed and constructed to facilitate cleaning and housekeeping.
2. A handwashing sink is available in the animal facility where infected animals are housed.
3. If the animal facility has windows that open, they are fitted with fly screens.
4. It is recommended but not required that the direction of air flow in the animal facility is inward and that exhaust air is discharged to the outside without being recirculated to other rooms.
5. An autoclave which can be used for decontaminating infectious laboratory waste is available in the building with the animal facility.

### ***Animal Biosafety Level 3***

#### **A. Standard Practices**

1. Doors to animal rooms open inward, are self-closing, and are kept closed when experimental animals are present.
2. Work surfaces are decontaminated after use or spills of viable materials.
3. Eating, drinking, smoking, and applying cosmetics are not permitted in the animal rooms.
4. Persons wash their hands after they handling cultures and animals and before leaving the laboratory.
5. All procedures are performed carefully to minimize the creation of aerosols.
6. An insect and rodent control program is in effect.

#### **B. Special Practices**

1. Cages are autoclaved before bedding is removed and before they are cleaned and washed.
2. Surgical-type masks or other respiratory protection devices (e.g. respirators) are worn by personnel entering animal rooms housing animals infected with agents assigned to Biosafety level 3.
3. Wrap-around or solid front gowns or uniforms are worn by personnel entering the animal room. Front-button laboratory coats are unsuitable. Protective must remain in the animal room and must be decontaminated before being laundered.
4. The laboratory or animal facility director restricts access to the animal room to personnel who have been advised of the potential hazard and who need to enter the room for program or service purposes when infected animals are present. 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 room.

5. The laboratory director or other responsible person establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific requirements (e.g. for immunization) may enter the animal room.
6. Hazard warning signs, incorporating the universal biohazard symbol is posted on the access door to the animal room containing animals infected with agents assigned to Biosafety level 3 are present. The hazard warning sign should identify the agent(s) in use, list the name and telephone number of the animal facility supervisor or other responsible persons(s), and indicates the special conditions of entry into the animal room (e.g., the need for immunizations or respirators).
7. Personnel wear gloves when handling infected animals. Gloves are removed aseptically and autoclaved with other animal room wastes before being disposed or reused.
8. All wastes from the animal room are autoclaved before disposal.
9. Hypodermic needles and syringes are used only for gavage or parenteral injection or aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable needle syringe units (i.e. the needle is integral to the syringe) are used for the injection or aspiration of infectious fluids. Needles should not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.
10. If floor drains are provided, the drain traps are always filled with water or a suitable disinfectant.
11. If vacuum lines are provided, they are protected with HEPA filters and liquid disinfectant traps.
12. Boots, shoe covers, or other protective footwear and disinfectant footbaths are available and used when indicated.

### C. Containment Equipment

1. Personal protective clothing and equipment and/or other physical containment devices are used for all procedures and manipulations of infectious materials or infected animals.
2. The risk of infectious aerosols from infected animals or their bedding can be reduced if the animals are housed in partial containment caging systems, such as open cages placed in ventilated enclosures (e.g., laminar flow cabinets) solid wall and bottom cages covered by filter bonnets, or other equivalent primary containment systems.

#### D. Animal Facilities

1. The animal facility is designed and constructed to facilitate cleaning and housekeeping and is separated for areas which are open to unrestricted personnel traffic within the building. Passage through two sets of doors is the basic requirement for entry into the animal room from access corridors or other contiguous areas. Physical separation of the animal room from access corridors or other activities may also be provided by a double doored clothes change room (showers may be included), airlock, or other access facility which requires passage through two sets of doors before entering the animal room.
2. The interior surfaces of walls, floors, and ceilings are water resistant so that they may be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate fumigation or space decontamination.
3. A foot, elbow, or automatically operated handwashing sink is provided near each animal room exit door.
4. Windows in the animal room are closed and sealed.
5. Animal room doors are self-closing and are kept closed when infected animals are present.
6. An autoclave which for decontaminating wastes is available, preferably within the animal room. Materials to be autoclaved outside the animal room are transported in a covered leakproof container.
7. An exhaust air ventilation system is provided. This system creates directional airflow that draws air into the animal room through the entry area. The building exhaust can be used for this purpose if the exhaust air is not recirculated to any other area of the building, is discharged to the outside, and is dispersed away from occupied areas and air intakes. Personnel must verify that the direction of the airflow (into the animal room) is proper. The exhaust air from the animal room that does not pass through biological safety cabinets or other primary containment equipment can be discharged to the outside without being filtered or otherwise treated.
8. The HEPA filtered exhaust air from Class I or Class II biological safety cabinets or other primary containment devices is discharged directly to the outside or through the building exhaust system. Exhaust air from these primary containment devices may be recirculated within the animal room if the cabinet is tested and certified at least every 12 months. If the HEPA filtered exhaust air from Class I or Class II biological safety cabinets is discharged to the outside through the building exhaust system, it is connected to this system in a manner (e.g., thimble unit connection) that avoids any interference with the air balance of the cabinets or building exhaust system.