

**Biosafety Manual  
for  
Louisiana State University Health Sciences Center-Shreveport**

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08/2004  
Date

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Date

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Chairperson of Biosafety Committee

\_\_\_\_\_  
Date

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## IMPORTANT TELEPHONE NUMBERS

### EMERGENCY TELEPHONE NUMBER

### IMPORTANT TELEPHONE NUMBERS

University Police	318-675-6165
Emergency	9-911
If using cellular phone	911
Facilities Services ( to report property damage or utility outage)	318-675-6319 or 318-675-4123
Safety Office	318-675-5410
Radiation Safety Office	318-675-5410
Biological Safety Office	318-675-5410
Occupation Health	318-675-8281
Shreveport Police Department	9-911
Louisiana State Highway Patrol	318-741-7411
Caddo Parish Sheriff's Office	318-675-2170
LSUHSC Switchboard	318-675-7001

### ASSISTANCE TELEPHONE NUMBERS

#### Department of Safety 55410

Hours: Monday thru Friday 8:00 am to 5:00 pm

After hours, weekends, and holidays call the Switchboard '0'

Biological Safety Officer 55410

Radiation Safety Officer 55410

#### Occupational Health

Occupational Health Clinic 5-6281 Hours: Monday thru Friday

8:00 am to 4:30 pm

After hours, Emergency Room located on 1<sup>st</sup> floor of the Hospital

## **POLICY STATEMENT**

### **Purpose**

This is an official policy statement of the Louisiana State University Health Sciences Center-Shreveport to establish the process for University compliance with the following documents:

***NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)***, current edition;  
<http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html>

***Biosafety in Microbiological and Biomedical Laboratories (BMBL)***, current edition.  
<http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm>

### **Policy**

Louisiana State University Health Sciences Center-Shreveport is actively committed to preserving the health and safety of its students, staff, and faculty, and to protecting the environment and the community. It is recognized that the use of potentially pathogenic microorganisms and organisms containing recombinant DNA (rDNA) is necessary in many University research and teaching laboratories. To ensure the safe handling of these organisms, the University requires compliance with the *NIH Guidelines* and with the recommendations outlined in the *BMBL*. Compliance with other applicable federal, state, and local regulations is also required.

### **Responsibilities**

**The Principal Investigator (PI):** is directly and primarily responsible for the safe operation of the laboratory. His/her knowledge and judgment are critical in assessing risks and appropriately applying the recommendations outlined in this manual. However, safety is a shared responsibility among all of the laboratory staff. Many resources exist to assist the PI with these responsibilities including, the Institutional Biosafety Committee (IBC), and the Department of Safety.

#### **A. The LSUHSC Department of Safety shall:**

Prepare the Biosafety Manual, with revisions as necessary

Distribute the Manual to each faculty member who works with biological materials

Investigate accidents involving infectious agents

Provide and/or coordinate biosafety training

Assist investigators with risk assessment and biosafety advice

The Safety Office shall work cooperatively with LSU researchers, faculty, and staff to promote biological and chemical safety on the campus.

**The PI shall:**

Assess the risks associated with laboratory experiments

Ensure the safe operation of their laboratory

Ensure that there is a list posted of contact personnel for emergency proposes

Train laboratory personnel in safe work practices (*This responsibility may not be shifted to inexperienced or untrained personnel.*)

Comply with all applicable state and federal regulations and guidelines

Register the following experiments with the IBC as required

- procedures involving recombinant DNA

- work with infectious agents

- experiments involving the use of human blood or other potentially infectious materials, such as unfixed human tissues, primary human cell lines, certain body fluids, and animal and plant pathogens

**The IBC shall:**

Review rDNA research conducted at or sponsored by the University for compliance with the *NIH Guidelines*, and approve those research projects that are found to conform with the *NIH Guidelines*;

Review research involving infectious agents conducted at or sponsored by the University for Compliance with the

guidelines in *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, and approve those research projects that are found to conform with the recommendations outlined in the *BMBL*.

Notify the PI of the results of the IBC's review and approval.

Report any significant problems with, or violations of, the *NIH Guidelines* and any significant research-related accidents or illness to the appropriate Institutional Official(s) and to the NIH Office of Biotechnology Activities (OBA) within 30 days.

Follow the guidelines for membership defined by the NIH.

**The Occupational Health Clinic shall:**

Provide medical surveillance, as required by the OSHA Bloodborne Pathogens Standard (CFR 1910.1030), and as recommended in the *BMBL* and *NIH Guidelines*.

Provide vaccinations, as required.

**Laboratory personnel shall:**

Comply with safety recommendations for the work being performed.

Report accidents or injuries to the PI and the LSU Safety Office.

**Deans/Department Chairs.**

Deans/Department Chairs are responsible for the implementation of safe practices and procedures in their respective Schools and/or Departments.

**Information**

The Safety Office will assist any Department in providing training and guidance for implementation of this policy.

## **Administrative Procedures Prior To Protocol Initiation**

Prior to submitting requests to the Institutional Biosafety Committee (IBC), contact the Safety Office to discuss biosafety issues and precautions.

Write specific Standard Operating Procedures (SOP's) for the planned procedures, which will be included in the IBC submission.

If animals and/or humans are to be used in the protocol, the Animal Care Committee/IRB will also have to grant approval for this protocol.

Designate the rooms where the adenovirus will be handled and the areas where post-inoculated animals will be housed. The Biological Safety Program will ensure that all rooms where virus administration and propagation is to take place are suitable.

Modifications to the protocol including room, agent or personnel changes must be submitted in writing to the Grants Office for IBC review and update.

All staff involved with the handling and administration of adenoviral vectors should receive Biosafety training that covers hazardous communication (HAZCOM) and safety procedures, before final IBC approval. It is the Principal Investigator's responsibility to identify the staff requiring this training, and to call the Safety Office to schedule a training session.

Respiratory protection is required for staff involved with handling and administration of adenovirus vectors outside of containment equipment (i.e. biological safety cabinets). Fit testing must be completed before final IBC approval. It is the Principal Investigator's responsibility to identify the staff requiring fit testing.

The IBC, which oversees recombinant DNA research at LSUHSC will review the registration documents.

### **Experiments covered by the *NIH Guidelines***

Some experiments involving rDNA molecules require registration and approval by the IBC before work may be initiated. Experiments that require IBC approval before initiation include those that:

use Risk Group 2, 3, 4, (see appendix) or Restricted Agents as host-vector systems.

use DNA from Risk Group 2, 3, 4, or Restricted Agents is cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector systems.

involve infectious virus, or defective virus in the presence of helper virus in tissue culture systems.

involve whole plants or animals.

involve more than 10 liters of culture.

Experiments that must be registered at the time of initiation include those involving:

the formation of recombinant DNA molecules containing no more than 2/3 of the genome of any eukaryotic virus propagated in tissue culture.

recombinant DNA-modified whole plants, and/or recombinant DNA-modified organisms associated with whole plants, except those that fall under Section III-A, III-B, III-C, or III-E of the *Guidelines*.

### **Experiments exempt from the *NIH Guidelines***

Experiments exempt from the *NIH Guidelines*, although requiring registration with the IBC, may be initiated immediately. The Chair of the IBC or the Safety Office will review the registration document and confirm that the work is classified correctly according to the *NIH Guidelines*. Exempt experiments are those that:

use rDNA molecules that are not in organisms or viruses.

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.

consist entirely of DNA from a prokaryotic host including its endogenous 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.

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).

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.

do not present a significant risk to health or the environment as determined by the NIH Director, with the advice of the Recombinant DNA Advisory Committee (RAC), and following appropriate notice and opportunity for public comment.

contain less than one-half of any eukaryotic viral genome propagated in cell culture.

Use *E. coli* K12, *Saccharomyces cerevisiae*, or *Bacillus subtilis* host - vector systems, unless genes from Risk Group 3 or 4 pathogens or restricted animal pathogens are cloned into these hosts.

## **CLASSIFICATION OF POTENTIALLY INFECTIOUS AGENTS**

Procedures and facilities involved in protecting laboratory workers, the public, and the environment from laboratory biological hazards are governed by federal and state regulations and guidelines. Many granting agencies require that grant recipients certify that they adhere to these guidelines and the regulations.

Each PI is responsible for registering all recombinant DNA experiments, including those exempt from the *NIH Guidelines*. The Safety Office audits all laboratories where BL2 or BL3 containment is required, as well as BL1 and clinical laboratories in the Medical School and Hospital.

## **Microorganisms**

The National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC) publish guidelines for work with infectious microorganisms. The publication, entitled *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, recommends that work be performed using one of four levels of containment: Biosafety Level 1 (BL1), BL2, BL3, and BL4. The *NIH Guidelines* (Appendix B) classify pathogenic agents into one of four risk groups according to specific criteria. It is LSUHSC policy that all laboratories adhere to these NIH/CDC guidelines.

### **Microorganisms capable of causing infection in humans**

Investigators must register any project involving a pathogenic agent with the IBC and receive its approval before work is begun. Following receipt of the completed Registration Document by Safety Office, the laboratory will be surveyed by the Safety Office to ascertain that it meets the containment requirements listed in *BMBL* for the agent being studied. If the lab meets the requirements, the work will be reviewed and approved or disapproved by the IBC.

### **Genetically Engineered Microorganisms**

Work with all genetically engineered organisms is to be done in compliance with the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*. These guidelines classify recombinant DNA experiments into four levels of containment (BL1, BL2, BL3, and BL4) based on the hazard of the microorganism and the procedures and quantities being used. It is LSUHSC policy that all laboratories follow these guidelines.

### **Select Agents**

The federal government, through the Department of Health and Human Services and the Department of Agriculture, regulates certain biological agents and toxins that are considered a threat to the public health, animal, or plant health and animal or plant products. Investigators must contact the Safety Office to register with the University's Select Agent Program and the appropriate federal agency prior to possession, use, or transfer of any Select Agent. See Select Agent Policy for more information.

## Human Clinical Materials

Please refer to the Bloodborne Pathogens Exposure Control Plan (Hospital Safety Manual or Chemical Laboratory Manual) for detailed information on handling human clinical material.

Work with human clinical material is regulated by the Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Standard, 29 CFR, Part 1910.1030. Human blood, unfixed tissue, and certain other body fluids are considered potentially infectious for bloodborne pathogens such as hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV). All human clinical material should be presumed infectious and handled using BL2 work practices. This concept is called "Universal Precautions". Investigators are responsible for notifying the Safety Office and Occupational Health Clinic of their use of human clinical materials so training and immunization can be provided as required by OSHA.

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## BIOSAFETY CONTAINMENT LEVELS

Four levels of biosafety are defined in the publication *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, published by the CDC and NIH. The levels, designated in ascending order by degree of protection provided to personnel, the environment, and the community, are combinations of laboratory practices, safety equipment, and laboratory facilities. Most microbiological work at Louisiana State University Health Sciences Center-Shreveport is conducted at BL1, BL2 and BL3 containment. There are no BL4 laboratories at the University. This manual covers standards for BL 1, BL 2, and BL 3 level laboratories.

### Principals of Biosafety; Containment

The term "containment" is used in describing safe methods for managing infectious agents in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other people, and the outside environment to potentially hazardous agents. The three elements of containment include laboratory practice and technique, safety equipment, and facility design.

**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 risk assessment of the work to be done with a specific agent will determine the appropriate combination of work practices, safety equipment, and facility design to provide adequate containment.

**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 handling such material safely. The PI or laboratory supervisor is responsible for providing or arranging for appropriate training of personnel.

Each laboratory should develop an operational manual which identifies specific 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 the handling of infectious agents must direct laboratory activities.

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

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

**Safety Equipment (Primary Barriers).** Safety equipment includes biological safety cabinets, enclosed containers (i.e., safety centrifuge tubes) and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The biological safety cabinet (BSC) is the principal device used to provide containment of infectious splashes or aerosols generated by many microbiological procedures. There are three types of biological safety cabinets used in microbiological laboratories see Appendix for description and illustrations. Safety equipment also may include items for personal protection such as personal protective clothing, respirators, face shields, safety glasses, or goggles. Personal protective equipment is often used in combination with other safety equipment when working with biohazardous materials. In some situations, personal protective clothing may form the primary barrier between personnel and the infectious materials.

**Facility Design (Secondary Barriers):** The design of a facility is important in providing a barrier to protect people working inside and outside the laboratory, and to protect people or animals in the community from infectious agents which may be accidentally released from the laboratory. Facilities must be commensurate with the laboratory's function and the recommended biosafety level for the agent being manipulated.

The recommended secondary barrier(s) will depend on the risk of transmission of specific agents. For example, the exposure risks for

most laboratory work in Biosafety Level 1 and 2 facilities will be direct contact with the agents, or inadvertent contact exposures through contaminated work environments. Secondary barriers in these laboratories may include separation of the laboratory work area from public access, availability of a decontamination facility (e.g., autoclave) and hand washing facilities.

When the risk for aerosol transmission increases, higher levels of primary containment and multiple secondary barriers may become necessary to prevent infectious agents from escaping into the environment. Such design features could include specialized ventilation systems to assure directional airflow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks at laboratory entrances, or separate buildings or modules for isolation of the laboratory.

## **BIOSAFETY LEVELS**

There are four biosafety levels (BSLs), which consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the operations performed the documented or suspected routes of transmission of the infectious agents, and for the laboratory function or activity. The recommended biosafety level for an organism represents the conditions under which the agent can be ordinarily handled safely. When specific information is available to suggest that virulence, pathogenicity, antibiotic resistance patterns, vaccine and treatment availability, or other factors are significantly altered, more (or less) stringent practices may be specified.

### **BIOSAFETY LEVEL 1**

BL 1 is the least restrictive and is suitable for work involving well-characterized agents not known to consistently cause diseases in healthy adult humans. BSL 1 laboratories are appropriate for undergraduate and secondary educational training as well as teaching laboratories. It represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for hand washing. See Appendix 1 for defined criteria

## **BIOSAFETY LEVEL 2**

BL2 is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists, (2) access to the laboratory is limited when work is being conducted, (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 biological safety cabinets or other physical containment equipment. With good microbiological techniques, work at BL2 can be conducted safely on the open bench, provided the potential for producing splashes or aerosols is low. Primary hazards to personnel working with BL2 agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials. BL2 is appropriate when work is done with any human-derived blood, body fluids, or tissues where the presence of an infectious agent may be unknown. See Appendix 2 for a complete list of BL2 criteria.

## **BIOSAFETY LEVEL 3**

BL3 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. Primary hazards to personnel working at BL3 relate to autoinoculation, ingestion, and exposure to infectious aerosols. See Appendix 3 for a complete list of BL3 criteria.

## **Clinical Laboratories**

Clinical laboratories, especially those in health care facilities, receive clinical specimens with requests for a variety of diagnostic and clinical support services. Typically, the infectious nature of clinical material is unknown, and specimens are often submitted with a broad request for microbiological examination for multiple agents (e.g., sputa submitted for "routine," acid-fast, and fungal cultures). It is the responsibility of the laboratory director to establish standard procedures in the laboratory that realistically address the issue of the infective hazard of clinical specimens.

Except in extraordinary circumstances (e.g., suspected hemorrhagic fever), the initial processing of clinical specimens and serological

identification of isolates can be done safely at Biosafety Level 2. This requires the use of Universal Precautions with all clinical specimens of blood or other potentially infectious material. Additionally, other recommendations specific for clinical laboratories may be obtained from the [National Committee for Clinical Laboratory Standards](#).

Biosafety Level 2 recommendations and OSHA requirements focus on the prevention of percutaneous and mucous membrane exposures to clinical material. Primary barriers such as biological safety cabinets (BSC) or plexiglass splash shields should be used when performing procedures that might cause splashing, spraying, or splattering of droplets. BSC's also should be used for the initial processing of clinical specimens when the nature of the test requested or other information suggests the likely presence of an agent readily transmissible by infectious aerosols (e.g., *M. tuberculosis*), or when the use of a BSC is indicated to protect the integrity of the specimen.

**The segregation of clinical laboratory functions and limited or restricted access to such areas is the responsibility of the laboratory director. It is also the director's responsibility to establish standard, written procedures that address the potential hazards and the required precautions to be implemented.**

## **ANIMAL FACILITIES**

Four biosafety levels are also described for activities involving infectious disease work with experimental mammals. These four combinations of practices, safety equipment, and facilities are designated Animal Biosafety Levels 1, 2, 3, and 4, (ABL1, ABL2, ABL3, ABL4), and provide increasing levels of protection to personnel and the environment. There are no ABSL4 laboratories at LSUHSC Contact Animal Resources for more information. See Appendix for more detailed information.

### **Risk Groups.**

Agents are classified into four Risk Groups (RGs) (see Appendix 1) according to their relative pathogenicity for healthy adult humans by the following criteria:

**Risk Group 1 (RG1)** agents are not associated with disease in healthy adult humans. These agents can be manipulated using Biosafety Level 1 procedures. Standard recombinant DNA experiments are typically classified as Biosafety Level 1.

**Risk Group 2 (RG2)** agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available. These agents can be manipulated using Biosafety Level 2 procedures.

**Risk Group 3 (RG3)** agents are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available. These agents shall be manipulated using Biosafety Level 3 procedures.

**Risk Group 4 (RG4)** agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available. These agents shall be manipulated using Biosafety Level 4 procedures.

## **Medical Surveillance**

A medical surveillance program will be provided through Occupational Medicine for those personnel having direct animal contact. All Departments must be compliant with Tuberculosis Control Resolutions for animal use.

It is the Departments responsibility to contact and schedule a yearly surveillance program. Those that fail to comply with Occupation Health guidelines; access to Animal Resources will be denied and the lab in which the personnel are from, the whole lab will be suspended. (See form).

Vaccines for which the benefits (levels of antibody considered to be protective) clearly exceed the risk (local or systemic reactions) will be offered to all clearly identify at-risk personnel, because immunoprophylaxis may provide an additional level of protection. It is the Principal Investigator's responsibility to ensure that laboratory personnel who work in Biosafety Level 2 or Biosafety Level 3 facilities receive the appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g. hepatitis B vaccine, TB skin test, Vaccinia (smallpox) vaccine, etc.) and periodic testing as recommended for the agent(s) being handled. Baseline serum

samples may be collected as appropriate. Additional serum specimens may be periodically collected, depending on the agents handled or the function of the laboratory. For more information, contact Occupational Health 5-6280 or the Safety Office at 5-5410.

## **Engineering Controls**

### **Biohazard Warning Signs and Posting**

Each laboratory must have a room sign that provides safety information to visitors and service personnel. Room signs must contain designations for all laboratory hazards in use within the laboratory (carcinogens, acutely toxic agents, reproductive hazards, biohazards, radioactive materials, lasers and magnetic fields). All areas and laboratories that contain biohazardous agents must be posted with the biohazard warning sign (Figure 1). The background must be red/orange in color with a black universal biohazard symbol and black lettering.



**Figure 1**

All equipment (centrifuges, water baths, cryogenic freezers, incubators, etc.) that comes in contact with biohazardous materials must be labeled with the universal biohazard symbol. Laboratories can order them from a vendor or call the Safety Office 55410.

### **Biological Safety Cabinets (BSCs)**

BSCs are designed to contain aerosols generated during work with infectious material through the use of laminar airflow and high efficiency particulate air (HEPA) filtration. All personnel must develop proficient lab technique before working with infectious materials in a BSC.

The biological safety cabinet (BSC) is designed to provide protection to the product, the user, and the environment when appropriate practices and procedures are followed. Three types of BSCs (Class I, II, and III) and the horizontal laminar flow cabinet are described below.

The common element to all classes of biological safety cabinets is the high efficiency particulate air (HEPA) filter. All Biological Safety Cabinets have HEPA filtered air. This filter removes particulates of 0.3 microns with an efficiency of 99.97%. However, it does not remove vapors or gases.

The biosafety cabinet requires regular maintenance and certification by a professional technician to assure that it protects you, your experiments, and the environment.

Each cabinet should be certified:

- when it is installed
- each time it is moved or repaired
- and least annually.

The Safety Office administers a program for **annual** certification of all biological safety cabinets at the university with no cost to the user. Contact the Safety Office at 55410 to confirm that your cabinet is included in this program.

Any repairs need to be reported to the Safety Office and the designated vendor will be contacted. Each department is responsible for payment of repairs. The vendor will submit an estimate for cost of repairs and PI/Department will approve or disapprove. If the BSC is not certified the cabinet must not be used.

**The Class I BSC** is suitable for work involving low to moderate risk agents, where there is a need for containment, but not for product protection. It provides protection to personnel and the environment from contaminants within the cabinet. The Class I BSC does not protect the product from "dirty" room air. It is similar in air movement to a chemical fume hood, but has a HEPA filter in the exhaust system to protect the environment. In many cases Class I BSCs are used specifically to enclose equipment (e.g., centrifuges, harvesting equipment), or procedures (e.g. cage dumping, aerating cultures or homogenizing tissues) with a potential to generate aerosols that may flow back into the room. (Figure 2)

**The Class II BSC** protects the material being manipulated inside the cabinet (e.g., cell cultures, microbiological stocks) from external contamination as well as meeting requirements to protect personnel and the environment. There are four types of Class II BSCs: Type A1, Type A2, Type B1 and Type B2. The major differences between the three types may be found in the percent of air that is exhausted or recirculated, and the manner in which exhaust air is removed from the work area. (Figure 3)

**The Class I BSC**

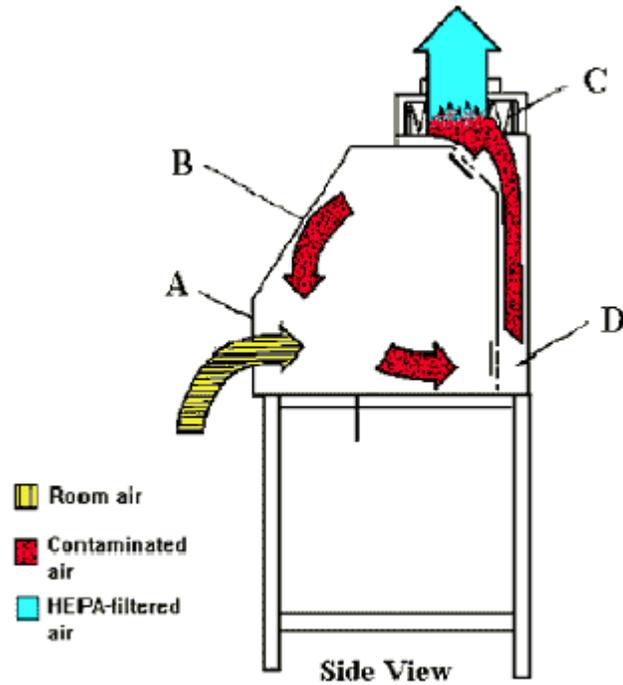


Figure 2

A. front opening B. sash C. exhaust HEPA  
D. exhaust plenum

## The Class II, Type A BSC

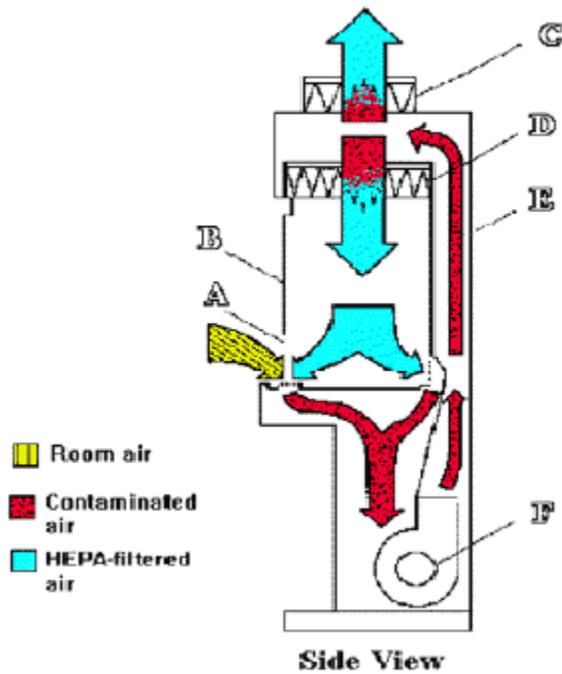


Figure 3

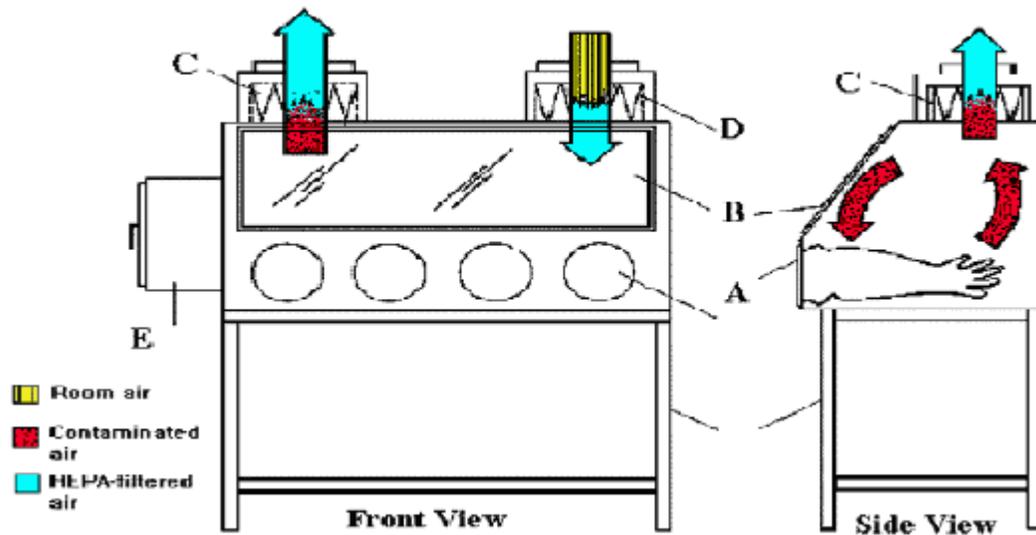
- A. front opening
- B. sash
- C. exhaust HEPA filter
- D. rear plenum
- E. supply HEPA filter
- F. blower

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## The Class III BSC

The gas-tight **Class III BSC**, or glove box, provides the highest attainable level of protection to personnel, the environment, and the product. It is the only cabinetry that provides a total physical barrier between the product and personnel. It is for use with *high-risk* biological agents and is used when absolute containment of highly infectious or hazardous material is required. (figure 4)

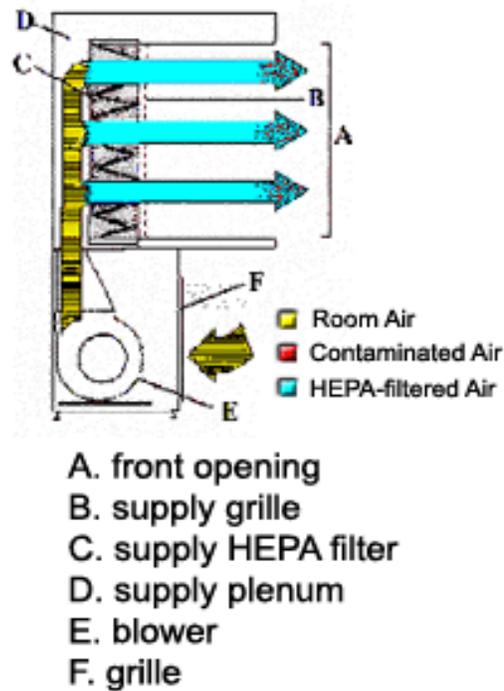
## The Class III BSC



**Figure 4**

- A. glove ports with O-ring for attaching arm-length gloves to cabinet
- B. sash
- C. exhaust HEPA filter
- D. supply HEPA filter
- E. double-ended autoclave or pass-through box

**Laminar Flow Clean Benches;** It is important to note that laminar flow clean benches must not be utilized for work with biohazardous or chemically hazardous agents. Clean benches provide product protection only, by ensuring that the product is exposed only to HEPA-filtered air. They do not provide protection to personnel or the ambient environment.



**Figure 5**

Horizontal laminar flow clean air benches are not biological safety cabinets. They discharge HEPA-filtered air across the work surface and toward the user. These devices only provide product protection. They can be used for certain clean activities, such as the dust-free assembly of sterile equipment or electronic devices. These benches should never be used when handling cell culture materials or drug formulations, or when manipulating potentially infectious materials. Horizontal laminar flow clean air benches are also inappropriate for use with uninfected animals (lab animal allergy), hazardous chemicals, and volatile radioisotopes. Horizontal laminar flow clean air benches should never be used as a substitute for a biological safety cabinet in research, biomedical, or veterinary laboratories.

## **Selection and Placement of Biosafety Cabinets in the Laboratory**

Certain considerations must be met to ensure maximum effectiveness of these primary barriers. Contact the Safety Office prior to purchase of a biosafety cabinet to ensure you have selected an appropriate unit for the proposed usage. Contact Physical Plant / Safety Office to ensure proper installation and placement of a biosafety cabinet in your laboratory space.

Adequate clearance should be provided behind and on each side of the cabinet to allow easy access for maintenance, and to ensure that the air return to the laboratory is not hindered. The ideal location for the biological safety cabinet is remote from the entry (i.e., the rear of the laboratory away from traffic), since people walking parallel to the face of a BSC can disrupt the protective laminar flow air curtain. The air curtain created at the front of the cabinet is quite fragile, amounting to a nominal inward and downward velocity of 1 mph. A BSC should be located away from open windows, air supply registers, or laboratory equipment (e.g., centrifuges, vacuum pumps) that creates turbulence. Similarly, a BSC should not be located close to a chemical fume hood.

### **Certification**

The operational integrity of a new BSC (biological safety cabinet) must be validated before it is put into service or after a cabinet has been repaired or relocated. Relocating a BSC may break the HEPA filter seals or otherwise damage the filters or the cabinet. Each BSC should be tested and certified at least annually to ensure continued proper operation.

Biological safety cabinets in laboratories and in animal care facilities approved for BSL2 experiments must be tested and certified annually by a qualified service person.

The Safety Office will annually recertify the BSC in the University. It is the responsibility of the Department or Principal Investigator to pay for repairs or relocation of a BSC (the cabinet will have to be recertified after relocation, the Department or Principal Investigator will be responsible for paying for certification). Contact the Safety Office for the current Contractor.

## Use of Open Flames

Manufacturer labels indicate that gas should not be plumbed into biological safety cabinets. This is due, in part, to the risk of gas from leaks becoming concentrated because of air recirculation in the cabinet.

Per CDC guidelines <http://www.cdc.gov/od/ohs/biosfty/bsc/bsc.htm> :

Open flames are not required in the near microbe-free environment of a biological safety cabinet. On an open bench, flaming the neck of a culture vessel will create an upward air current which prevents microorganisms from falling into the tube or flask. An open flame in a BSC, however, creates turbulence which disrupts the pattern of HEPA-filtered air supplied to the work surface. When deemed absolutely necessary, touch-plate microburners equipped with a pilot light to provide a flame on demand may be used. Internal cabinet air disturbance and heat buildup will be minimized. The burner must be turned off when work is completed. Small electric "furnaces" are available for decontaminating bacteriological loops and needles and are preferable to an open flame inside the BSC. Disposable sterile loops can also be used.

In addition to fire and gas leak hazards, the use of an open flame in a biological safety cabinet causes air turbulence altering airflow patterns that may disrupt the protective air barrier. Use of a flame should not be a substitute for good aseptic technique. Shielded electric incinerators or hot bead sterilizers are good alternatives to open flames to sterilize surgical instruments, biological loops, and needles. **Note:** Be aware that UV lights can cause gas line tubing to deteriorate and present a gas leak hazard.

## UV Lights

Many biological safety cabinets are equipped with ultraviolet (UV) lights. However, if good practices are followed, UV lights are not needed to protect tissue culture or other work. UV radiation should not take the place of wiping down the cabinet interior with a disinfectant or the practice of good aseptic technique. If you wish to use UV lamps, acquaint yourself with their limitations and hazards.

UV is effective only when it directly hits a microbial cell. Cells/spores may be protected from UV radiation by dust or organic matter. Likewise, UV lamps must be cleaned regularly to remove any dust and dirt that may block its germicidal effectiveness. Turn

off the light and wipe it with 70% ethanol every two weeks. Lights need to be replaced periodically. The length of time a lamp will be effective depends on the number of hours it is in use. Lamps should be checked periodically with a meter to ensure that the appropriate intensity of UV light is being emitted.

UV light does not penetrate cracks or seams so will not disinfect the spill area under the work surface - a favorite hideout for fungal spores.

Turn off UV light when the room is occupied - UV exposure can burn corneas and cause skin cancer.

Be aware that UV lights can cause gas line tubing to deteriorate and present a gas leak hazard.

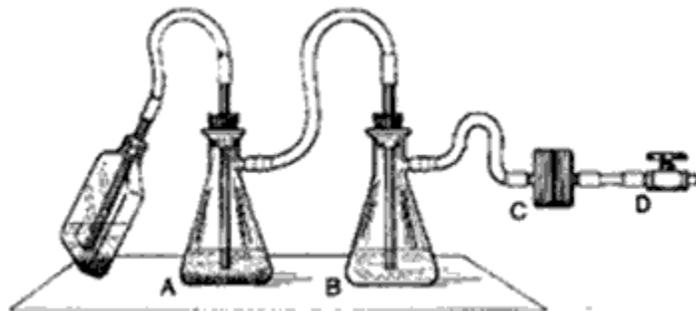
## **WORKING IN THE BIOLOGICAL SAFETY CABINET**

### **Vacuum Lines**

All vacuum lines should be protected from contamination and fluid intake. Vacuum Line Filter:

A hydrophobic filter will prevent fluid and aerosol contamination of central vacuum systems or vacuum pumps. The filter will also prevent microorganisms from being exhausted by a vacuum pump into the environment. Hydrophobic filters are available from several scientific supply companies.

### **Protecting the Vacuum System**



The above arrangement protects vacuum systems during aspiration. The left suction flask (A) is used to collect fluids into a suitable decontamination solution; the right flask serves as a fluid overflow collection vessel. A glass spargers in flask B minimizes splatter. An

in-line HEPA filter (C) is used to protect the vacuum system (D) from aerosolized materials.

Inactivation of aspirated materials can be accomplished by placing sufficient chemical decontamination solution into the flask to kill microorganisms as they are collected. Once inactivation occurs, liquid materials can be disposed of as noninfectious waste. Decontamination solution should be changed frequently.

All vacuum lines used to aspirate supernatants, tissue culture media, and other liquids that may contain microorganisms should be protected from contamination by the use of a collection flask and overflow flask. In addition, at BL2 and above, a hydrophobic vacuum line filter should be used.

### **Collection and Overflow Flasks**

Collection tubes should extend at least 2 inches below the sidearm of the flask.

Locate the collection flask inside the biosafety cabinet instead of on the floor, so the liquid level can be seen easily and the flask emptied before it overflows.

If a glass flask is used at floor level, place it in a sturdy cardboard box or plastic container to prevent breakage by accidental kicking.

In BL2 and BL3 laboratories, the use of Nalgene flasks is recommended to reduce the risk of breakage.

### **Safe & Effective Work Practices**

#### Before work is started:

Remove all unnecessary equipment and supplies from the cabinet. Clutter alters air flow. Check that the air grilles are clear.

Turn on the blower to remove particulates in the cabinet. Wait at least two to three minutes before using the cabinet.

Wipe down surface of cabinet interior with disinfectant.

Prepare a checklist of materials necessary for the activity.

Place supplies and needed equipment in the BSC before beginning work to minimize the number of arm-movement disruptions across the air barrier of the cabinet. Only items required for the immediate work should be placed in the BSC.

Place absorbent towels and decontaminating solution near the cabinet to facilitate quick clean up of spills.

Wipe the exterior of supplies with a disinfectant, particularly containers removed from a water bath. Segregate items that will remain clean from the ones that may become contaminated.

Wash hands and arms, wear appropriate protective equipment for the work being done and to prevent skin flora from contaminating your work.

Adjust stool height so that your neck and face are above the sash opening. The sash should be set at armpit level.

#### While working in the cabinet:

In order to prevent air disturbances that can breach the air barrier, never have more than one person at a time use a cabinet - even six-foot cabinets.

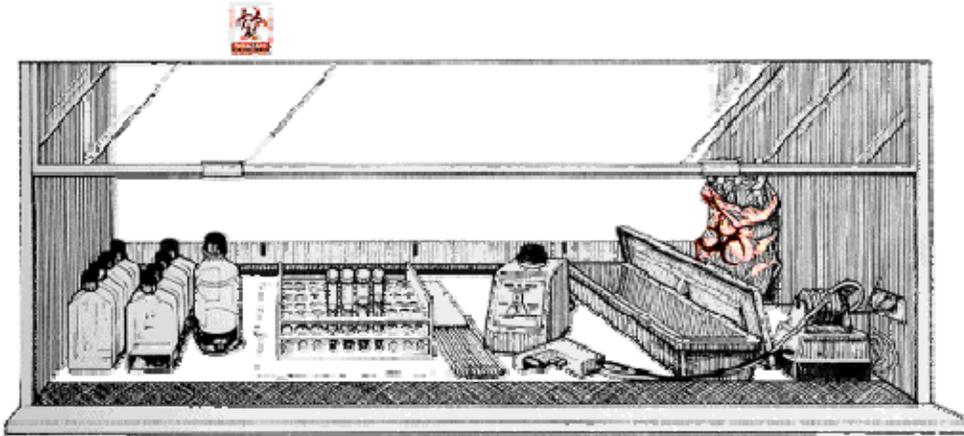
Delay manipulation of materials for approximately one minute after placing the hands/arms inside the cabinet.

Do not rest arms on the front grille. Raising arms slightly will lessen disruption of air flow.

Work as far back in the cabinet as practical - at least four inches inside the front grill edge.

Move arms slowly and limit arm movement in and out of cabinet. Do not swing side to side, but straight in and out.

As a general rule of thumb, keep clean materials at least one foot away from aerosol-generating activities to minimize the potential for cross-contamination. The work flow should be from "clean (left) to contaminated or dirty (right)". Limit the movement of "dirty" items over "clean" ones.



Not pouring liquids will eliminate the need to flame bottlenecks.  
Remove media with vacuum and replace with serological pipettes.

After work is complete:

Wipe down the surfaces of all containers and equipment with an appropriate disinfectant and remove from the cabinet.

Wipe down the cabinet interior with disinfectant.

Leave blower on for several minutes with no activity so that any airborne contaminants will be purged from the work area.

Remove gloves and wash hands.

Turn blower off and pull sash down. Wipe outside of sash with disinfectant.

Read Operators Manual for detailed instructions.

## **Spill Cleanup**

For small spills in a biological safety cabinet:

Leave the cabinet running.

Wipe down all supplies and equipment in cabinet.

Wipe down all interior cabinet surfaces with appropriate disinfectant.

For moderate spills in a biological safety cabinet:

Leave the cabinet running.

Cover spill area with paper towels.

Pour disinfectant over towels from edges of spill to center, be careful not to splatter.

Allow 20-30 minutes of contact time.

Determine if spill has gone beyond the work surface such as on the grilles or in side seams. If yes, disassemble as much of cabinet as possible for decontamination.

If the cabinet has a catch basin below the work surface that may be involved in the spill, flood the basin with disinfectant. Do not use alcohol as a large quantity of alcohol presents a flammable hazard. Clean basin after 20 minutes. Follow the manufacture's recommendations for decontamination procedures.

Autoclave or wipe down all items in cabinet with disinfectant.

Wipe down all interior surfaces.

Let cabinet run for at least 10 minutes after cleanup.

Place gloves and all clean up materials in red biohazard bag for pick up or clear autoclavable bag and autoclave for 60 minutes at 121 degrees Centigrade (256 degrees Fahrenheit).

Wash hands.

For major spills in a biological safety cabinet:

Contact the Safety Office at 55410 to determine if professional decontamination is indicated.

**BIOLOGICAL SPILLS**

A spill kit should be kept in each laboratory where work with microorganisms is conducted. Basic equipment is: concentrated disinfectant (such as chlorine bleach), a package of paper towels, gloves, autoclave bags, sharps container, and forceps to pick up broken glass

## **GENERAL SPILL CLEANUP GUIDELINES**

Wear PPE; gloves and lab coat.

Use forceps to pick up broken glass and discard into SHARPS container.

Cover spilled material with paper towels. Starting from the outside of the spill and go towards the center.

Add diluted disinfectant in sufficient quantity to ensure effective microbial inactivation.

Dispose of towels in biohazard waste container.

Wipe spill area with diluted disinfectant.

Remove PPE and dispose properly (biohazard box)

Wash hands with soap and water when finished.

## **SPECIFIC SPILL CLEANUP GUIDELINES**

### **Spill of BL2 material**

Keep other workers out of the area to prevent spreading spilled material. Post warning sign, if needed.

Remove contaminated clothing and put into a biohazard bag for decontamination later.

Wash hands and exposed skin and inform the PI of the spill. Call the Safety Office at 5-5410 for assistance, if necessary.

Put on protective clothing (lab coat, gloves and if needed, face protection and shoe covers) and assemble clean-up materials (disinfectant, autoclavable container or bag, forceps, sharps container, and paper towels).

Pick up broken glass with forceps and dispose into SHARPS container.

Cover the spill with paper towels and add appropriately diluted disinfectant.

After at least 20 minutes contact time, pick up the paper towels and re-wipe the spill area with diluted disinfectant.

Collect all contaminated materials into biohazard waste container and autoclave.

Remove PPE and dispose of properly.

Wash hands with soap and water.

### **Spill of a BL3 material**

Stop work immediately.

Avoid inhaling airborne material while quickly leaving the room. Notify others to leave. Close door, and post with warning sign.

Remove contaminated clothing, turn exposed area inward, and place in a biohazard bag. Wash hands with soap and water.

Notify the PI. Call the Safety Office at 55410 after hours and weekends call the Switchboard (dial '0'), they will contact the Safety Officer who is on call.

Allow 30 minutes to one hour for aerosols to disperse before re-entering the laboratory to begin clean-up.

Put on personal protective equipment (HEPA filtered respirator, gown, gloves, and shoe covers) and assemble clean-up materials (disinfectant, autoclavable container or bag, forceps, sharps container, and paper towels).

Contain the spill with absorbent paper towels or disposable pads. Carefully add 10% chlorine bleach to the spill; avoid creating aerosols when pouring the disinfectant. Leave the room and allow 30 minutes for the bleach to inactivate the material.

Pick up broken glass with forceps and discard in SHARPS container.

Clean up liquid with paper towels and collect all contaminated materials into biohazard bag or container. Remove all spilled materials and decontaminate the area again with an appropriate disinfectant.

Autoclave (or soak in 10% bleach solution) lab coat, gloves, and other protective equipment that was worn for clean up.

Wash hands thoroughly with soap and water.

## **INJURY INVOLVING BIOLOGICAL MATERIALS:**

**Laboratories using biological material should always have a MSDS (if available), Standard Operating Procedure (SOP), or Medical Surveillance written and in place, for the agent in use. These should be readily available to all lab personnel in case of exposure, and should be taken to Occupational Health or the Emergency room.**

### **For Severe Injuries**

Call University Police 5-6561/911 for assistance and transportation to the nearest emergency room.

Accompany the injured person to the medical facility and provide information to personnel about the accident/exposure.

Report accident to the PI and Safety Office.

### **For Splash to the Eye**

Immediately flush the eye with a gentle stream of clean, temperate water for 15 minutes. Hold the eyelid open. Be careful not to wash the contaminant into the other eye. Use emergency eyewash if one is accessible.

Contact the Occupational Health Clinic to obtain care. If it is closed, go to the Emergency Room located on the first floor of the hospital. Give as much information possible to medical staff, if there is a MSDS/SOP/Medical Surveillance for the biological agent, take it with you.

Report the accident to the PI and Safety Office, and seek additional medical assistance if necessary.

## **For Contamination to the Body**

Immediately remove contaminated clothing and drench skin with water. Wash with soap and water, and flush the area for 15 minutes.

Contact the Occupational Health Clinic to obtain care. If clinic is closed, go to the Emergency Room located on the first floor of the hospital.

Report the injury to the PI and to the Safety Office, and seek additional medical assistance if necessary.

## **FIRES INVOLVING BIOLOGICAL MATERIALS**

**Without placing yourself in danger**, put biological materials in secure location, such as incubator or freezer.

Activate the building fire alarm.

Call Security/Safety Office to notify what room fire is located

Leave the building at once and go to your designated meeting area for your department

Have person with knowledge of the incident meet the fire department outside and give information.

## **DECONTAMINATION AND DISPOSAL**

Sterilization, disinfection, and antisepsis are all forms of decontamination.

**Sterilization** implies the killing of all living organisms.

**Disinfection** refers to the use of antimicrobial agents on inanimate objects; its purpose is to destroy all non-spore forming organisms.

**Antisepsis** is the application of a liquid antimicrobial chemical to living tissue.

## CHEMICAL DISINFECTANTS

Chemical disinfectants are used to render a contaminated material safe for further handling, whether it is a material to be disposed of as waste, or a laboratory bench on which a spill has occurred. It is important to choose a disinfectant that has been proven effective against the organism being used. Chemical disinfectants are registered by the EPA under the following categories:

Sterilizer or Sterilant - will destroy all microorganisms including bacterial and fungal spores on inanimate surfaces.

Disinfectant - will destroy or irreversibly inactivate specific viruses, bacteria, and pathogenic fungi, but not bacterial spores.

Hospital Disinfectant - agent shown to be effective against *S. aureus*, *V. cholerae* and *P. aeruginosa*. It may be effective against *M. tuberculosis*, pathogenic fungi or specifically named viruses.

Antiseptic - agent formulated to be used on skin or tissue - not a disinfectant.

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## DISINFECTANTS COMMONLY USED IN THE LABORATORY

### Iodophors

Recommended dilution is 75 ppm, or approximately 4.5 ml/liter water.

Effective against non-spore forming bacteria, fungi, and viruses.

Effectiveness reduced by organic matter (but not as much as with hypochlorites).

Stable in storage if kept cool and tightly covered.

Built-in color indicator; if solution is brown or yellow, it is still active.

Relatively harmless to humans.

## Hypochlorites (bleach)

Working dilution is 1: 10 to 1: 100 in water.

Effective against non-spore forming bacteria, fungi, most viruses at 1: 100 dilutions.

Effective against bacterial spores at 1: 10 dilution.

Very corrosive.

Rapidly inactivated by organic matter.

Solutions decompose rapidly; fresh solutions should be made daily.

## Alcohols (ethanol, isopropanol)

The effective dilution is 70-85%.

Effective against a broad spectrum of bacteria and many viruses.

Fast acting, leaves no residue.

Non-corrosive.

Not effective against bacterial spores.

## Important Characteristics of Disinfectants

	Hypochlorites "Bleach"	Iodoform "Wescodyne"	Ethyl Alcohol
Shelf-life > 1 wk	X	X	
Corrosive	X	X	
Residue	X	X	
Inactivation by organic matter	X	X	
Skin Irritant	X	X	
Respiratory Irritant	X	X	
Eye Irritant	X	X	X
Toxic	X	X	x

## DILUTION OF DISINFECTANTS

### Chlorine compounds (Household Bleach)

Bleach solutions decompose at room temperature and should be made fresh daily. However, if stored in tightly closed brown bottles, bleach solutions retain activity for 30 days. The use concentration is dependent on the organic load of the material to be decontaminated. Use a 1% solution to disinfect clean surfaces, and 10% solution to disinfect surfaces contaminated with a heavy organic load. To disinfect liquid biological waste before disposal, add concentrated bleach to a final concentration of 1%.

### Iodophor

Manufacturer's recommended dilution is 3 ounces (90 ml) into 5 gallons water, or approximately 4.5 ml/liter. For porous surfaces, use 6 ounces into 5 gallons water.

### Alcohols

Ethyl alcohol and isopropyl alcohol diluted to 70 - 85% in water are useful for surface disinfection of materials that may be corroded by a halogen or other chemical disinfectant. Use 70-85% alcohol (more is not better) because the higher percent of alcohol the more it becomes a preservative.

## AUTOCLAVING

**It is the responsibility of each department to ensure that all users understand and know how to use the autoclave correctly.**

Researchers who work with potentially infectious materials are at a higher risk of exposure, especially when exposed to untreated infectious waste. Infection may be transmitted through several different routes, including contact with untreated infectious waste, indirect contact with contaminated instruments or environmental surfaces, or inhalation of airborne contaminants.

The Occupational Safety and Health Association (OSHA) relies on guidelines published by the Center for Disease Control and Prevention (CDC) as a widely recognized and accepted standard to be followed by employers in carrying out their responsibilities under the Occupational Safety and Health Act. The CDC and OSHA recommend the use Biological Indicators (BI) for monitoring steam sterilization cycles in

autoclaves. The CDC states [for medical autoclaves] “proper functioning of sterilization cycles should be verified by the periodic use (at least weekly) of biological indicators (i.e., spore tests). Heat-sensitive chemical indicators (e.g., those that change color after exposure to heat) alone do not ensure the adequacy of sterilization cycle.” (CDC, 2004).

Autoclaves use pressurized steam to destroy microorganisms, and are the most dependable system available for the decontamination of laboratory waste and the sterilization of laboratory glassware, media, and reagents. For efficient heat transfer, steam must flush the air out of the autoclave chamber. Before using the autoclave, check the drain screen at the bottom of the chamber and clean if blocked. If the sieve is blocked with debris, a layer of air may form at the bottom of the autoclave, preventing efficient operation.

### **Container Selection**

**Polypropylene bags.** Commonly called biohazard or autoclave bags, these bags are tear resistant, but can be punctured or burst in the autoclave. Therefore, **place bags in a rigid container during autoclaving.** Bags are available in a variety of sizes, and some are printed with an indicator that changes color when processed.

Polypropylene bags are impermeable to steam, and for this reason should **not** be twisted and taped shut, but gathered loosely at the top and secured with a large rubber band or autoclave tape. This will create an opening through which steam can penetrate.

**Polypropylene containers and pans.** Polypropylene is a plastic capable of withstanding autoclaving, but resistant to heat transfer. Therefore, materials contained in a polypropylene pan will take longer to autoclave than the same materials in a stainless steel pan. To decrease the time required to sterilize material in these containers,

remove the lid (if applicable).

turn the container on its side when possible.

select a container with the lowest sides and widest diameter possible for the autoclave.

**Stainless steel containers and pans.** Stainless steel is a good conductor of heat and is less likely to increase sterilizing time, though is more expensive than polypropylene.

### **Preparation and Loading of Materials**

Fill liquid containers only half full.

Loosen caps, or use vented closures.

The autoclave bag should never be over-packed or sealed too tightly.

Keep contents of the autoclave bag at a minimum.

Always put bags of biological waste into pans to catch spills.

Position biohazard bags on their sides, with the bag neck taped loosely.

Leave space between items to allow steam circulation.

Household dishpans melt in the autoclave. Use autoclavable polypropylene or stainless steel pans.

### **Cycle Selection**

Use liquid cycle (slow exhaust) when autoclaving liquids, to prevent contents from boiling over.

Select fast exhaust cycle for glassware.

Use fast exhaust and dry cycle for wrapped items.

### **Time Selection**

Take into account the size of the articles to be autoclaved. A 2-liter flask containing 1 liter of liquid takes longer to sterilize than four 500 ml flasks each containing 250 ml of liquid.

Material with a high insulating capacity (animal bedding, high-sided polyethylene containers) increases the time needed for the load to reach sterilizing temperatures.

Bags of biological waste should be autoclaved for 50 minutes to ensure decontamination.

## Removing the Load

Check that the chamber pressure is zero.

Wear lab coat, eye protection, heat insulating gloves, and closed-toe shoes.

Stand behind door when opening it.

Slowly open door only a crack. Beware of rush of steam.

After the slow exhaust cycle, open autoclave door and allow liquids to cool for 10-20 minutes before removing.

## Monitoring

Autoclaves used to decontaminate laboratory waste should be tested periodically to assure effectiveness. Two types of tests are used: 1) a chemical indicator that fuses when the temperature reaches 121°C, and 2) heat-resistant spores (*Bacillus stearothermophilis*) that are killed by exposure to 121°C for approximately 15 minutes. Both types of tests should be placed well down in the center of the bag or container of waste, at the point slowest to heat.

The chemical test should be used first to determine that the temperature in the center of the container reaches 121°C. Ampoules of heat-resistant spores should be used in subsequent test runs to determine the amount of time necessary to achieve sterilization.

If you need assistance, please contact the Safety Office 55410.

## RECORD KEEPING

The use of an autoclave logbook is recommended for each autoclave. Users must fill in all information prior to autoclaving.

The logbook should be located adjacent to the autoclave and maintained by the department.

Information that should be part of the logbook should include:

User's name

Cycle time

Cycle setting

Materials being autoclaved

Contact number

Time in

Time out

User's department

## **SAFETY MAINTENANCE**

General maintenance should be conducted on an annual basis or as recommended by the manufacture. Specifically, the safety valve should be checked and replaced as required. A maintenance logbook should be maintained. Each user should always conduct a quick check prior to each use to ensure that all parts are properly working. Appropriate cleaning protocol should be obtained from each manufacture.

## **TRAINING**

Each autoclave user should be trained on the proper use of the autoclave. The cycle settings should be posted next to the autoclave to inform each user of the types of settings available, such as gravity and liquid cycles, the temperatures for each cycle, and run times.

A written sterilization procedure should be kept near each autoclave and a standard operating procedure should be developed. It should include:

The appropriate sterilization times for liquid and dry goods.

Identification of standard treatment containers

Proper load placement procedures

Personal protection equipment required for removing materials from the autoclave

Instructions for loading and unloading

Instructions on cleaning and maintaining the autoclave

## **USE AND DISPOSAL OF SHARPS:**

To prevent needle stick injuries:

Avoid using needles whenever possible.

Use safety needles/syringes whenever possible. PI must have a written plan stating reason for unable to use safety syringes.

Do not bend, break, or otherwise manipulate needles BY HAND.

Do not recap needles BY HAND. Do not remove needles from syringes BY HAND.

Immediately after use, discard needle and syringe (whether contaminated or not) into puncture resistant sharps containers.

Never discard sharps into regular trash.

**Never discard sharps into bags of biological waste.**

Use care and caution when cleaning up after procedures that require the use of syringes and needles.

Do not overfill the sharps containers. Close completely (tape shut) when they are 3/4 full and request pickup or you may place them in the Biohazard box.

Locate sharps containers in areas in which needles are commonly used. Make containers easily accessible.

Sharps containers may be purchased from Central Medical Supply, located in the hospital. Well as from laboratory supply distributors such as VWR and Fisher Scientific.

### **In the event of a needle stick injury:**

Allow to bleed freely.

Wash thoroughly with soap and water.

Notify supervisor and go immediately to Occupational Health. If closed, go to the Emergency Room located on the first floor of the hospital.

Fill out report

# **BIOLOGICAL WASTE DISPOSAL PROCEDURES**

## **I. Biological Waste**

All biological waste from BL1, BL2, and BL3 laboratories must be decontaminated prior to disposal.

Decontamination and disposal are the responsibility of the person/laboratory generating the waste.

Collect disposable, solid materials contaminated by an infectious agent, **excluding sharps, or broken or unbroken glass**, into an autoclave bag within a sturdy container. When full, these bags are autoclaved, cooled, and then placed in a biohazard box, and picked up by Environmental Services – Housekeeping 56337. Biohazard boxes are then transported to be incinerated. DO NOT exceed 40 lbs.

Decontaminated liquids containing a biological agent by the addition of a chemical disinfectant such as sodium hypochlorite (household bleach) or an iodophor, **or** by autoclaving, then dispose of by pouring down the sink. **It is not necessary to autoclave liquids that have been chemically disinfected.**

**NOTE:** However, if bleach has been used in the tray used to collect labware that will later be autoclaved, sodium thiosulfate must be added to the bleach to prevent the release of chlorine gas during autoclaving.

## **II. Reusable Labware**

Items such as culture flasks and centrifuge bottles are decontaminated by lab personnel before washing by one of two methods.

Autoclave items that have been collected in autoclavable container.

Chemically disinfect items by soaking in diluted disinfectant for one hour before washing with soap and water.

## **III. Disposal of Blood Products and Body Fluids**

All human blood and other potentially infectious materials should be handled using Universal Precautions.

Discard disposable items contaminated with human blood or body fluids (**excluding sharps and glassware**) into the incinerator containers that are available from Environmental Services – Housekeeping 56337. Do not overfill containers or use without the plastic liners provided with them. These containers may be used for temporary storage and accumulation of waste. When full, close and seal the plastic liner. Containers are NOT to exceed 40 lbs.

Biological waste pickup. Environmental Services – Housekeeping 56337 will collect and dispose of all containers.

#### **IV. Disposal of Sharps and Disposable Glassware**

Discard all needles, needle and syringe units, scalpels, and razor blades, **whether contaminated or not**, directly into rigid, red/white, labeled sharps containers. Do not recap, bend, remove, or clip needles. Sharps containers should not be overfilled. To request pickup of sharps containers, call Environmental Services at 56337. Alternatively, closed sharps containers may be packaged in biohazard containers (Section III above). Sharps containers may be purchased from Central Medical Supply.

**Uncontaminated (Clean glass only)** Pasteur pipets and broken or unbroken glassware are discarded into containers specifically designed for broken glass disposal, or into heavy-duty cardboard boxes that are closeable. When boxes are full, call Environmental Services 56337 for pick-up. Pasteur Pipets can also be placed into Sharps Containers

**Contaminated** pasteur pipets, and broken or unbroken glassware may be treated in one of two ways:

Discarded into approved sharps containers, as in Section A above, or

Decontaminated by autoclaving or chemical disinfection, and then discarded into glass disposal boxes as in Section B above.

Sharps that are contaminated with radioactive materials or hazardous chemicals should be discarded into separate sharps containers labeled with the name of the isotope or chemical. Contact the Safety Office 55410 for disposal information.

## **V. Multi-hazard or Mixed Waste**

Avoid generating mixed waste if possible. Keep volume to minimum.

Never put laboratory waste into office waste containers

Do not autoclave mixed waste.

When discarding waste containing an infectious agent and radioactive material, inactivate the infectious agent first, and then dispose of as radioactive waste. Seek advice from the RSO at 55410 before beginning inactivation procedures.

When discarding waste containing an infectious agent and a hazardous chemical, inactivate the infectious agent first, and then dispose of as chemical waste. Seek advice before beginning inactivation procedures. Contact the Safety Office at 55410 for instructions.

## **VI. Disposal of Animal Tissues, Carcasses, and Bedding**

Disposal of animal carcasses/tissues is coordinated through the Animal Resource Facility (ARF).

Place animal carcasses/tissues into plastic bag. Double-bag when carcass contains zoonotic agent (transmissible from animals to humans).

Place bag in freezer located in the ARF on the ninth floor.

Disposal of animal carcasses/tissues that are contaminated with radioactive materials or hazardous chemicals call Safety Office or Assistant Director of Animal Resources for directions.

## **VII. Disposal Containers**

Each laboratory is responsible for purchasing containers for the disposal of biological waste, EXCEPT that biohazard boxes (with liners) will be provided by Environmental Services. The following types of containers are available:

Sharps containers may be purchased from Central Medical Supply as well as from laboratory product distributors. They are available in various sizes, and should be puncture resistant, red, labeled as "Sharps," and have a tightly closing lid. Do not purchase "needle-cutter" devices, which may produce aerosols when used.

Biohazard Autoclave Bags may be purchased from various laboratory product distributors, such as Fisher Scientific or VWR. Be sure to select polypropylene bags which are able to withstand autoclaving. They should be placed inside a rigid container with lid while waste is being collected.

Biohazard Containers are provided by Environmental Services. A plastic liner (also provided by Environmental Services) must be used to prevent contamination of the box.

Glass Disposal Boxes may be purchased from various laboratory product distributors. Alternatively, heavy-duty, closeable cardboard boxes may be used for disposal of broken glass. Small glass boxes may be placed in the biohazard box as long as the weight of the biohazard box does not exceed 40 lbs.

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## **VIII. What to do with Filled Waste Containers**

Sharps containers and incinerator boxes – Lab personnel can place Sharps Container, taped closed in the biohazard box. Call Environmental Services for pick up.

Biohazard autoclave bags and glass disposal boxes - close and autoclave bags, tape boxes closed; Call Environmental Services for pick up and Safety Office for Glass box pick up.

## **CENTRIFUGE CONTAINMENT**

Examine centrifuge tubes and bottles for cracks or stress marks before using them.

Never overfill centrifuge tubes since leakage may occur when tubes are filled to capacity. Fill centrifuge tubes no more than 3/4 full.

Make sure load is balanced.

Tightly seal all centrifuge tubes or use safety cups/ buckets to prevent aerosol escape.

Take care that matched sets of buckets, adapters, and plastic inserts are kept together.

Ensure that rotors are "locked" to the spindle and buckets are "seated" before operation.

Use a biological safety cabinet (BSC) to load and open tubes, safety cups, and buckets when working with biohazardous materials. Decontaminate tubes, safety cups, and buckets before removal from the BSC and transport to the centrifuge.

Close the centrifuge top during operation.

Allow the centrifuge to come to a complete stop before opening.

Disinfect weekly and immediately following any spill or breakage the surfaces of the centrifuge head, bowl, trunnions, and buckets. Use 70% alcohol, 2% glutaraldehyde, or any registered mycobactericidal. For radioactive contamination, use equal parts of 70% ethanol, 10% SDS, and water, followed by water rinses and drying with a soft cloth. DuPont COUNT- OFF and other radioactive decontaminates must not be used on aluminum rotors as they will remove the anodized coatings. Contact the Safety Office for more information.

**Ultra Centrifuges (In addition to the above):**

1. Clean rotors, lids, adapters, and associated parts with 1% non-alkaline detergent, rinse with distilled water, and dry with a soft cloth. Encrusted material should be removed with a twist bristle brush and 1% non-alkaline soap solution.
2. Lubricate weekly all O-rings with vacuum grease and metal rotor threads with antigalling grease.
3. Make sure that rotors are locked to the spindle and that buckets are properly seated on their pins. Only use the rotor handle tool to tighten ultra speed lids.
4. Do not use rotors which have been dropped or struck against a hard surface.
5. Contact your centrifuge representative for specific information.

6. A log book must be kept for ultra centrifuge rotors as the hours run determine the life of the rotor.

7. All new users of centrifuges must be trained by an appointed instructor (who may be an appropriately qualified or experienced member of the laboratory staff) before attempting to use a centrifuge.

## **SHIPMENT OF BIOLOGICAL MATERIALS**

### **GENERAL INFORMATION**

Shipment of infectious agents, biological products, and clinical specimens is regulated by many agencies, and requirements are not always uniform. In addition, regulations are continually modified and new ones are added. A summary of current requirements is presented here, but it is recommended that the investigator check with the various agencies before shipping any material that may be regulated.

In general, **first** determine whether the material you wish to ship requires a permit, and begin the application process, if required. **Second**, decide on a carrier, and learn the packaging and labeling requirements of that carrier.

### **PACKAGING**

Various carriers (FedEx, UPS, Postal Service or others) have different requirements for packaging and labeling infectious substances. In addition, various agencies such as the International Air Transport Association (IATA), and the Department of Transportation (DOT) have developed guidelines and procedures to facilitate the safe shipment of infectious substances. Therefore, it is important to check with the carrier you have chosen to determine their specific requirements for shipping infectious agents. In addition to the materials listed above that require permits, the following materials are likely to require special packaging and/or labeling:

Infectious Substance a viable microorganism, or its toxin, which causes or may cause disease in humans.

Clinical Specimen any human or animal material including blood, tissue, and tissue fluids, shipped for the purpose of diagnosis.

Biological Product a product for human or veterinary use, such as vaccines and investigational new drugs.

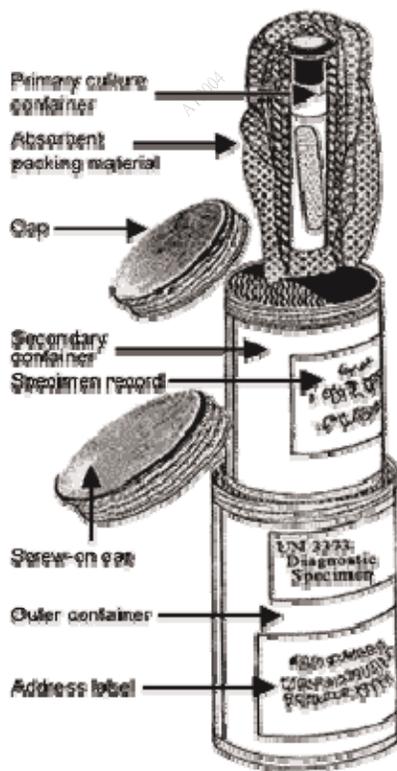
The basic component of all shipping requirements, with various minor modifications, is triple packaging, as follows:

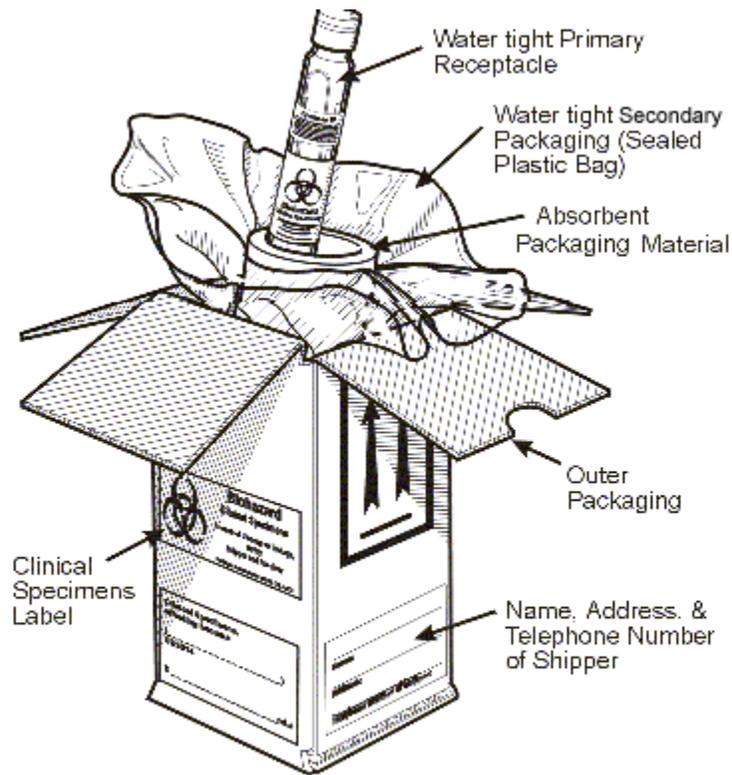
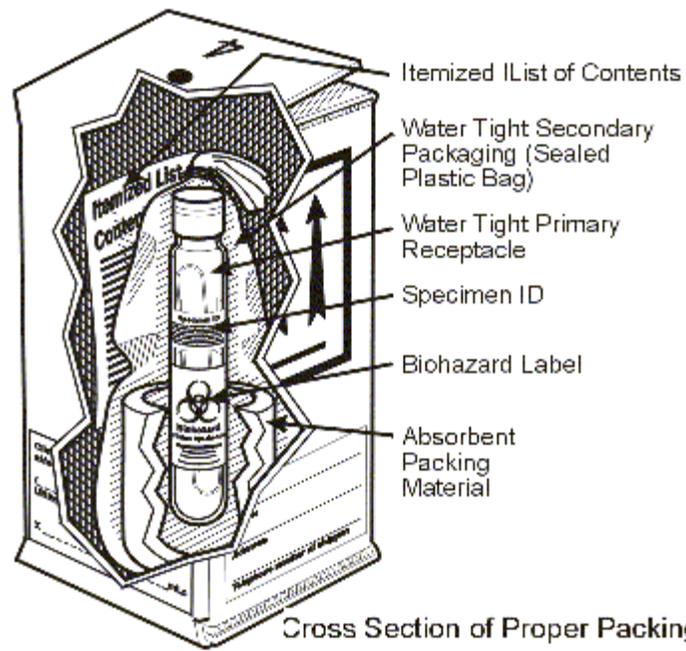
A primary container that contains the specimen;

A secondary container that contains the primary container and packaging capable of absorbing the specimen;

An outer rigid shipping container that contains the secondary container and other material.

NOTE: All personnel shipping infectious or biological materials must have a record of training for the transportation of dangerous goods. Call the Safety Office for more information 5-5410.





## **GENETICALLY MODIFIED MICROORGANISMS**

The *NIH Guidelines for Experiments Involving Recombinant DNA Molecules* (April 2002) states that:

Host organisms should be shipped as etiologic agents, regardless of whether they contain recombinant DNA (rDNA), if they are regulated as human pathogens, animal pathogens, or plant pests.

Host organisms should be shipped as etiologic agents if they contain 1) rDNA that includes the complete genome of an organism that is a human or animal pathogen or plant pest; 2) rDNA that codes for a toxin involved in eliciting human, animal, or plant disease, and is carried on an expression vector or within the host chromosome; or 3) rDNA from an organism regulated as a human or animal pathogen or a plant pest that has not been adequately characterized.

## **HUMAN CLINICAL MATERIAL**

The OSHA Bloodborne Pathogens Standard requires that all packages containing human blood and other potentially infectious materials be labeled with the universal biohazard symbol or color coded. Various carriers may have additional requirements.

## **ON CAMPUS TRANSPORT BETWEEN LABORATORIES OR BUILDINGS**

When moving infectious substances between labs or buildings on campus, the following minimum procedures must be followed:

Always use a leak-proof sealed primary container within a leak-proof sealed secondary container.

All samples must be in sealed primary container. Utilize plastic containers whenever possible.

Place primary container in sealed secondary container, with absorbent (paper towels) between primary and secondary container suitable for the volume transported.

If dry ice is needed, the secondary container should be placed in an outer container, with the dry ice placed between the secondary and tertiary container (never place dry ice in a sealed container).

Place biohazard label with agent name, lab number, and phone number on outer container.

Leave the material with a known responsible individual in the receiving lab. Do not leave the material unattended or with an unknown individual.

Infectious material or biological toxins can not be transported on buses or in private vehicles.

Regardless no opened container with research samples may be transported through the Medical School without the samples being in a secondary closed container.

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## APPENDIX 1

### **Biosafety in Microbiological and Biomedical Laboratories 4<sup>th</sup> Edition, May 1999 Centers for Disease Control and Prevention and National Institutes of Health**

#### **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 biological safety cabinet is 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.

## **APPENDIX 2**

### **Biosafety Level 2**

BIOSAFETY LEVEL 2 is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists, (2) access to the laboratory is limited when work is being conducted, (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 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:

#### **Standard Microbiological Practices**

Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.

Persons wash their hands after they handle viable materials and animals, after removing gloves, and before leaving the laboratory.

Eating, drinking, smoking, handling contact lenses, and applying cosmetics 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 for this purpose only.

Mouth pipetting is prohibited; mechanical pipetting devices are used.

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

Work surfaces are decontaminated at least once a day and after any spill of viable material.

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 at off-site from the laboratory are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.

An insect and rodent control program is in effect.

### **Special Practices:**

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 be unusually hazardous are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at 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.

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

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.

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).

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.

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.

The laboratory director ensures that laboratory 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.

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

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.

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.

Syringes which re-sheath the needle, needle-less systems, and other safe devices should be used when appropriate.

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.

Cultures, tissues, or specimens of body fluids are placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping.

Laboratory equipment and work surfaces should be decontaminated with an appropriate 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.

Spills and accidents which 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.

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

### **Safety Equipment (Primary Barriers)**

Properly maintained biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are used whenever:

Procedures with a potential for creating infectious aerosols or splashes are conducted. 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 intranasal, and harvesting infected tissues from animals or eggs.

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 biological safety cabinet.

Face protection (goggles, mask, faceshield or other splatter guards) 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.

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.

Gloves are worn when handling infected animals and when hands may contact 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.

### **Laboratory Facilities (Secondary Barriers)**

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

Consider locating new laboratories away from public areas.

Each laboratory contains a sink for handwashing. Foot, knee, or automatically operated sinks are recommended.

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

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

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.

Install biological safety cabinets in such a manner that fluctuations of the room supply and exhaust air do not cause the biological safety cabinets to operate outside their parameters for containment. Locate biological safety 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 biological safety cabinets' air flow parameters for containment.

An eyewash facility is readily available.

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

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.

## **APPENDIX 3**

### **Biosafety Level 3**

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 materials are conducted within biological safety cabinets or other physical containment devices, or by personnel wearing appropriate personal protective clothing and equipment. The laboratory has special engineering and design features.

It is recognized, however, that many existing facilities may not have all the facility safeguards recommended for 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 Biosafety Level 2 facilities. However, the recommended Standard Microbiological Practices, Special Practices, and Safety Equipment for Biosafety Level 3 must be 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:

### **Standard Microbiological Practices**

Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.

Persons wash their hands after handling infectious materials and animals, after removing gloves, and when they leave the laboratory.

Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the laboratory. 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 for this purpose only.

Mouth pipetting is prohibited; mechanical pipetting devices are used.

Policies for the safe handling of sharps are instituted.

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

Work surfaces are decontaminated at least once a day and after any spill of viable material.

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. Infectious waste from BSL-3 laboratories should be decontaminated before removal for off-site disposal.

All Minors are prohibited from working or conducting research in the following:

Any laboratory or facility designated as BSL-3, ABSL-3 or higher for recombinant or infectious organisms

Any laboratory where select agents or explosives are used or stored.

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

### **Special Practices:**

Laboratory doors are kept closed when experiments are in progress.

The laboratory director controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. 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 risk of acquiring infections. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. No minors will be allowed in the laboratory,

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. (See Select Agent Policy)

When infectious materials or infected 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.

Laboratory personnel receive the appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing), and periodic testing as recommended for the agent being handled.

Baseline serum samples are collected and stored for all laboratory and other at-risk personnel. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the laboratory. An initial baseline then annual afterwards.

A biosafety manual specific to the laboratory is prepared or adopted by the laboratory director and precautions are incorporated into standard operating procedures. Personnel are advised of special hazards and are required to read and to follow instructions on practices and procedures.

Laboratory 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 changes.

The laboratory director is responsible for insuring that, before working with organisms at Biosafety Level 3, all personnel demonstrate proficiency in standard microbiological practices and techniques and in the practices and operations specific to the laboratory facility. This might include prior experience in handling human pathogens or cell cultures, or a specific training program provided by the laboratory director or other competent scientist proficient in safe microbiological practices and techniques.

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

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.

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.

Syringes which re-sheath the needle, needle-less systems, and other safe devices should be used when appropriate.

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 should be decontaminated before disposal.

All manipulations involving infectious materials 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.

Laboratory equipment and work surfaces should be decontaminated with an appropriate disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination with infectious materials.

Spills of infectious materials are decontaminated, contained, and cleaned up by appropriate professional staff, or others properly trained and equipped to work with concentrated infectious material. Spill procedures are developed and posted.

Contaminated equipment must be decontaminated before removal from the facility for repair or maintenance or packaging for transport.

Cultures, tissues, or specimens of body fluids are placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping.

All potentially contaminated waste materials (e.g., gloves, lab coats, etc.) From laboratories or animal rooms are decontaminated before disposal or reuse.

Spills of infectious materials are decontaminate, contained, and cleaned up by appropriate professional staff, or others properly trained and equipped to work with concentrated infectious material.

Spill and accidents which result in overt or potential exposures to infectious materials are immediately reported to the PI and Safety Office. Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained.

Animals and plants not related to the work being conducted are not permitted in the laboratory.

The need for and type of respiratory protection is based on the research being conducted. The investigator should discuss respiratory protection equipment with the Safety Office.

### **Safety Equipment** (Primary Barriers)

Protective laboratory clothing such as solid-front or wrap-around gowns, scrub suits, or coveralls are worn by workers when in the laboratory. Protective clothing is not worn outside the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when overtly contaminated.

Gloves must be worn when handling infectious materials, infected animals, and when handling contaminated equipment.

Frequent changing of gloves accompanied by hand washing is recommended. Disposable gloves are not reused.

All manipulations of infectious materials, necropsy of infected animals, harvesting of tissues or fluids from infected animals or embryonate eggs, etc., are conducted in a Class II or Class III biological safety cabinet (see Appendix A).

When a procedure or process cannot be conducted within a biological safety cabinet, then appropriate combinations of personal protective equipment (e.g., respirators, face shields) and physical containment devices (e.g., centrifuge safety cups or sealed rotors) are used.

Respiratory and face protection are used when in rooms containing infected animals.

### **Laboratory Facilities (Secondary Barriers)**

The laboratory is separated from areas which are open to unrestricted traffic flow within the building. Passage through two sets of self-closing doors is the basic requirement for entry into the laboratory from access corridors or other contiguous areas. A clothes change room and shower is included in the passageway.

Each laboratory contains a sink for handwashing. The sink is hands-free or automatically operated and is located near the laboratory exit door.

The interior surfaces of walls, floors, and ceilings are water resistant so that they can be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate decontamination. Seams, if present, must be sealed. Walls, ceilings, and floors should be smooth, impermeable to liquids and resistant to the chemicals and disinfectants normally used in the laboratory.

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

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.

All windows in the laboratory are closed and sealed.

A method for decontaminating all laboratory wastes is available, preferably within the laboratory (i.e., autoclave, chemical disinfection, incineration, or other approved decontamination system). Consideration should be given to means of decontaminating equipment. If waste is transported out of the laboratory, it should be properly decontaminated, sealed, and not transported in public corridors.

Biological safety cabinets are required and are located away from doors, from room supply louvers, and from heavily-traveled laboratory areas.

A ducted exhaust air ventilation system is provided. This system creates directional airflow that draws air from "clean" areas into the laboratory toward "contaminated" areas. The exhaust air is not recirculated to any other area of the building, and is discharged to the outside with filtration and other treatment optional. The outside exhaust must be dispersed away from occupied areas and air intakes. Laboratory personnel must verify that the direction of the airflow (into the laboratory) is proper.

The High Efficiency Particulate Air (HEPA)-filtered exhaust air from Class II or Class III biological safety cabinets is discharged directly to the outside or through the building exhaust system. If the HEPA-filtered exhaust air from Class II or III biological safety cabinets is to be discharged to the outside through the building exhaust air system, it is connected to this system in a manner (e.g., thimble unit connection) that avoids any interference with the air balance of the cabinets or building exhaust system. Exhaust air from Class II biological safety cabinets may be recirculated within the laboratory if the cabinet is tested and certified at least every twelve months.

Continuous flow centrifuges or other equipment that may produce aerosols are contained in devices that exhaust air through HEPA filters before discharge into the laboratory. These HEPA systems are tested at least annually. Alternatively, the exhaust from such equipment may be vented to the outside if it is dispersed away from occupied areas and air intakes.

Vacuum lines are protected with liquid disinfectant traps and HEPA filters, or their equivalent, which are routinely maintained and replaced as needed.

An eyewash facility is readily available inside the laboratory.

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

The Biosafety Level 3 facility design and operational procedures must be documented. The facility must be tested for verification that the design and operational parameters have been met prior to operation. Facilities should be re-verified, at least annually, against these procedures as modified by operational experience.

Additional environmental protection (e.g., personnel showers, HEPA filtration of exhaust air, containment of other piped services and the provision of effluent decontamination) should be considered if recommended by the agent summary statement, as determined by risk assessment, the site conditions, or other applicable federal, state, or local regulations

Repair and Preventive Maintenance- Repair or routine preventive maintenance of mechanical or laboratory equipment in posted biohazard areas are not to be initiated without prior clearance from the Safety Office.

Removal of equipment – Potentially contaminated equipment is not to be removed from biohazard laboratories for repair, servicing, cleaning or to surplus or any other areas until decontamination and removal of biohazard labels have been performed. The investigator is to certify such equipment as being free of biohazard agents. Service personnel may ask laboratory personnel to sign a waiver stating that the piece of equipment has been appropriately decontaminated.

**NOTE:** Biosafety level 3 agents should not be handled when service personnel are in the laboratory to minimize potential exposure to them.

## APPENDIX 4

### Laboratory Security and Emergency Response for Microbiological and Biomedical Laboratories

Traditional laboratory biosafety guidelines have emphasized the use of good work practices, appropriate containment equipment, well designed facilities, and administrative controls to minimize risks of accidental infection or injury for laboratory workers and to prevent contamination of the environment outside the laboratory.

Although clinical and research microbiology laboratories may contain a variety of dangerous biological, chemical, and radioactive materials, there are few reports to date of any of those materials being used intentionally to injure laboratory workers or others.

However there is a growing concern about the possible use of biological, chemicals, and radioactive materials as agents for terrorism. In response to these concerns, the following guidelines address laboratory security issues (e.g., preventing unauthorized entry to laboratory areas and preventing unauthorized removal of dangerous biological agents from the laboratory).

The following are offered for laboratories **using biological agents or toxins capable of causing serious or fatal illness to humans or animals**. Most of these laboratories would be working under the BSL-2 or BSL-3 conditions describes in Section II and III. However, research, clinical, and production laboratories working with newly identified human pathogens, high-level animal pathogens, and/or toxins not covered by BSL-2 or -3 recommendations, should also follow these guidelines to minimize opportunities for accidental or intentional removal of these agents from the laboratory.

1. Recognize that laboratory security is related to but different than laboratory safety.
  - a. Review safety policies procedures regularly
  - b. Laboratory supervisors should ensure that all laboratory workers and visitors understand security requirements and are trained and equipped to follow established procedures.
  - c. Review policies and procedures whenever an incident occurs or a new threat is identified.
2. Control access to areas where biological agents or toxins are used and stored.

Laboratory and animal care areas should be separated from the public areas of the buildings in which they are located. Laboratory and animal care areas should be locked at all times.

Card-keys or similar devices should be used to permit entry to laboratory and animal care areas.

All entries (including entries by visitors, maintenance workers, repairmen and others needing one-time or occasional entry) should be recorded, either by the card-key device (preferable) or by signature in a log book.

Only workers required to perform a job should be allowed in laboratory areas, and workers should be allowed only in areas and at hours required to perform their particular job.

Access for student, visiting scientists, ect., should be limited to hours when regular employees are present.

Access for routine cleaning, maintenance, and repairs should be limited to hours when regular employees are present (BSL 3 laboratories)

Freezers, refrigerators, cabinets, and other containers where stocks of biological agents, hazardous chemicals, or radioactive materials are stored should be locked when they are not in direct view of workers (e.g., when located in unattended storage areas).

3. Know who is in the laboratory area

- a. All workers should be known to facility administrators and laboratory directors. Depending on the biological agents involved and the type of work being done, a background check and/or security clearance may be appropriate before new employees assigned to the laboratory area.
- b. All workers (including students, visiting scientists, and other short-term workers) should wear visible identification badges. Identification badges should include, at a minimum, a photograph, the wearer's name, and an expiration date. Guests should be issued identification badges, and escorted or cleared for entry using the same procedures as for regular workers.

4. **Know what material are being brought into the laboratory area.**

- a. All packages should be screened (visual) before being brought into the laboratory area.
- b. Packages containing specimens, bacterial or virus isolates, or toxins should be opened in a safety cabinet or other appropriate containment device.

**5. Know what materials are being removed from the laboratory area.**

- a. Biological materials/toxins for shipment to other laboratories should be packaged and labeled in conformance with all applicable local, federal, and international shipping regulations.
- b. Required permits (e.g., PHS, DOT, DOC, USDA) should be in hand before materials are prepared for shipment.
- c. The recipient (preferably) or receiving facility should be known to the sender, and the sender should make an effort to ensure that materials are shipped to a facility equipped to handle those materials safely.
- d. Hand-carrying of microbiological materials and toxins to other facilities is rarely appropriate. If biological materials or toxins are to be hand carried on common carriers, all applicable regulations must be followed. (See Infectious Shipping Manual)
- e. Contaminated or possible contaminated materials should be decontaminated before they leave the laboratory area.
- f. Chemicals and radioactive materials should be disposed of in accordance with local, state, and federal regulations.

**6. Have an emergency plan.**

Control of access to laboratory areas can make an emergency response more difficult. This must be considered when emergency plans are developed.

An evaluation of the laboratory area by appropriate facility personnel, with outside experts if necessary, to identify both safety and security concerns should be conducted before an emergency plan is developed. Facility administrators, laboratory directors, principal investigators, laboratory workers, the facility safety office, and facility security officials should be involved in emergency planning.

Police, fire, and other emergency responders should be informed as to the types of biological materials in use in the laboratory areas, and assisted in planning their responses to emergencies in the laboratory areas.

Plans should include provisions for immediate notification of (and response by) laboratory directors, laboratory workers, safety office personnel, or other knowledgeable individuals when an emergency occurs, so they can deal with biosafety issues if they occur.

Laboratory emergency planning should be coordinated with facility-wide plans. Such factors as bomb threats, severe weather (hurricanes, floods), earthquakes,

power outages, and other natural (or unnatural) disasters should be considered when developing laboratory emergency plans.

**7. Have a protocol for reporting incidents.**

Laboratory directors, in co-operation with facility safety and security officials, should have policies and procedures in place for reporting and investigation of incidents or possible incidents (e.g., undocumented visitors, missing chemicals, unusual or threatening phone calls).

## **APPENDIX 5**

### **SECURITY PLAN**

Security plan must be reviewed on an annual basis or when there is a change in the plan, i.e., staff change, breach of security, new treat, disgruntled employee, etc.

All staff must be specifically trained for the specific security needs of the specific laboratory or facility.

The biological laboratory must be a controlled access. Laboratories that have select agents must be kept secured at all times. All laboratory doors and select agent storage areas must have limited access (authorized entry only) and kept locked at all times when no authorized laboratory personnel are present.

All laboratory specific staff must know who authorized users are. Access for students, visitors, routine cleaning, maintenance and repairs, should be limited to hours when regular employees are present. This can be accomplished with an identification badge. (BSL 3 laboratories)

Approach any visitor that appears to be wandering in the laboratory area and ask if you can help direct them. Non-essential, non-routine, known visitor should check-in and check out.

Lock all equipment (e.g., freezers, cabinets, incubators, etc.) that may contain biological or chemical hazards. This is mandatory as a condition of regulated imported commodities.

Keep laboratory entry doors closed at all times. Lock and secure laboratory when no authorized individual is present. Even for a short time, i.e., bathroom breaks, running to the main office, etc. After normal business hours, all laboratories must be locked when not in use. Laboratory building exterior doors are secured after normal business hours. To minimize the likelihood of unauthorized access, all after-hours building users should:

Avoid providing building access to unfamiliar individuals.

Secure doors behind you, acknowledge that door is secured and locked.

Immediately report any building security problem to Campus Security.

Research or other activities involving the use of lab space, materials or equipment without the knowledge and approval of the responsible Principal Investigator is strictly prohibited. Violation of this prohibition may result in disciplinary action up to and including termination.

Post emergency contacts, including responsible person, a second person knowledgeable with the laboratory, and a 24-hour contact number. Keep information current (must be annually reviewed).

Inventory of biological commodities must be current and updated annually.

Know what materials are being removed or entered in the laboratory.

## **LOSS, THEFT AND VANDALISM**

Any incident of inventory or facility must be reported directly to Security.

Undocumented visitors, unusual or threatening phone calls, or violent incident.

Bomb threats, severe weather, earthquakes, power outages, other natural or unnatural disaster, fire should be coordinated with the specific laboratory emergency plan.

Suspicious activities: signs of tampering with equipment or facilities, suspicious materials or devices, and misplaced equipment.

Lost, missing, or unsolicited parcel.

## **HANDLING SUSPICIOUS PACKAGES**

### **Mail Screening**

Staff responsible for incoming mail should maintain an awareness of the possibility of suspicious letters and packages (parcels).

No return or fictitious address.

Note place of origin (delivery is uncommon) – foreign, Priority Special Delivery.

Handwritten or poorly typed addresses.

Misspelling of common words.

Restrictive mailings such as "**confidential**," "**personal**", "to **be opened by addressees only**," etc.

Excessive weight (unbalanced or weight is excessively heavy for its volume) and/or a feel of powdery substances.

Rigid, lopsided, or uneven envelopes.

Excessive postage.

Excessive binding material; masking, electrical, or strapping tape, string, twines.

Incorrect titles.

Protruding wires, screws, or other metal parts.

Titles but no names.

Oily stains or discoloration.

Irregular sizes and stiffness.

No knowledge of sender or unrequested (unsolicited).

Inner enclosures.

CDC Select Agents and Toxin must have accompanying authorization.

If a parcel or letter exhibits any of these warning clues follow the University protocol for accepting damaged or leaking parcel or follow the recommendation below:

### **Unopened Parcel**

**DO NOT OPEN IT.** There is no risk if the parcel is not leaking or damaged.

Call Campus Security.

Avoid handling the package or keep handling to a minimum.

Make the parcel safe. Initiate facility's contingency plan. Place in a clear or transparent plastic bag (zip lock or trash bag) seal.

### **Damaged or Leaking Parcel**

Avoid handling of parcel. Don appropriate Personal Protective Equipment (PPE): gloves, safety glasses, and dust mask.

Inspect adjacent parcels for potential contamination and put aside any that may have been contaminated from the leaking or damaged parcel.

Place all leaking, damaged, or contaminated parcels in a clear or transparent plastic bag (zip lock or trash bag) seal.

Call campus security.

### **Opened Parcel**

Place all content back into original parcel.

Place in a clear or transparent plastic bag (zip lock or trash bag) seal.

Stay in the room and notify Campus Security. (Do not contaminate others or other areas). **STAY CALM.**

Person that opened the parcel and others who had direct contact with parcel must wash hands, immediately with soap and water.

### **Unsolicited Parcel**

Do not accept. Return to sender.

Verify with sender what the content of the parcel is.

## **CHAIN OF CUSTODY**

All documentation must be accounted for accordingly when there is transporting, movement, or shipping. It is recommended that carefully maintained records be maintained with adequate security. This record keeping is necessary to help investigators, in the event that an incident occurs. But availability of these records to unauthorized should be carefully limited (sensitive information).

## **FACILITIES**

Access controls are intended to prevent the entry of unauthorized individuals to the restricted laboratory. Employees, authorized individuals with access privileges must take an active role to prevent unknown or unauthorized persons from going into the restricted facilities.

1. Control of access to laboratory areas can make an emergency response more challenging. This must be considered when emergency plans are developed.
2. Have a protocol for reporting incidents. Laboratory directors, in cooperation with facility safety and security officials, should have policies and procedures in place for the reporting and investigation of incidents or possible incidents, such as undocumented visitors, missing chemicals, or unusual or threatening phone calls.
3. Review and update if necessary the lab's emergency contact information on your door signs, located on or near your laboratory door.

## **RESPONSIBILITIES**

### **All staff**

Should know who works in their area and have a general understanding of other areas and groups in their facility and department.

Respect access control measures.

Must ensure that their visitors, vendors, contractors, and temporary personnel are aware of and follow access guidelines.

Challenge anyone that is not authorized.

Report any lost or stolen property immediately, call Security.

Should not lend access keys or code to others not authorized.

Report to supervisor if they become a target of hate mail, phone calls, demonstrations, and treats of violence either at work or home.

## **Management**

Hold employees and non-employees accountable for using good access procedures.

Challenge those staff members who do not comply to the access control guidelines.

Consult security to determine the appropriate access control measures.

Immediately communicate to Campus Security job transfers that require the revoking of access privileges.

## **Non-Employees**

Visitors, consultants, and contractors without access privileges. Challenging unauthorized individuals. Simply introduce yourself and ask where the person works or who he/she is looking for. Ask contractors where the project is located. Verify information. Call Safety Office, Security, or BRI. In case of suspicious behavior, contact Campus Security immediately.

## **CYBER SECURITY**

Insure computer security and the continuity and integrity of computer based records. Restrict access to computers and adhere to password and data backup procedures, store a copy off site. Establish procedures for power loss. Protect data with reliable anti-virus software. Secure computer work areas, and computers within these areas. Protect wiring from environmental or intentional damage.

Computers shall not have references to sensitive information on their hard drives. All files should be stored on transferable media (floppy, zip drives, and CD) system and secured. Any loss or compromise of computer software or hardware with respect to sensitive information shall be reported to University Police. When disposing of outdated computers, all hard drives must be assured of complete erasure of any sensitive information.

## **GENERAL SECURITY CONTINGENCY PLANS**

Plans must be in place. Plans for secured areas should include means for emergency responders to gain access to the work area in the event of emergency. Tours of the work area by emergency response organizations should be considered for high hazard locations.

The following emergency procedures are recommended if there is fire, explosions, or other laboratory accidents. This procedure is intended to limit injuries and minimizes damage if an accident should occur.

Render assistance to persons involved and remove them from exposure to further injury if necessary and capable; do not move an injured person not in danger of further harm.

Warn personnel in adjacent areas of any potential hazard to their safety.

Render immediate first-aid (e.g., beginning resuscitation if breathing has stopped; help washing under a safety shower).

In case of fire, call the Safety Office and University Police.

In a medical emergency, summon medical help immediately (Medical School and BRI 9-911). Ideally laboratories without medical staff should have personnel trained in first-aid available during working hours.

Have appropriate first aid kit in laboratory and know how to use it.

### **Fire and Explosion**

The doors to each area should be properly labeled with the highest level of physical containment required for work in that area.

Each piece of equipment used for storing, processing, or handling viable biological materials should be labeled with the universal biohazard symbol. Each of these items should have emergency telephone numbers attached.

In the event of a fire or explosion, implement the following in addition to those steps outlined in the site emergency response plan:

Place all biohazards material in an incubator, refrigerator, or freezer.

Turn off all gas outlets.

In the event of a gas leak, implement the following in addition to those steps outlined in the site emergency response plan:

Control ignition sources.

Call Safety Office, Security if your lab is located in the BRI call 5-4100.

Turn off gas if location of valve is known and safe to do so.

If significant, evacuate and keep people out.

**Bomb Threat:** More information can be found in the **Hospital Safety Manual Policy 2.11**

Bomb Threats can be received by telephone, mail, or anonymous e-mail.

### **Receiving a Bomb Treat**

When a threats is received by phone:

#### **Stay calm**

Prolong the call by asking questions to gather as much information as possible regarding the threat and the caller.

Write down the information about the threat and caller.

Notify University Police as soon as possible

Do not discuss the threats with non-emergency personnel.

### **When a threat is received by mail**

Save all materials including any envelope or container(s).

Avoid any unnecessary handling.

Report the threat to Security.

Do not discuss the threat with non-emergency personnel.

### **When a threat is received by e-mail**

Save the messages.

Report the threat to Security.

Do not discuss the threat with non-emergency persons.

### **Response to a Bomb Threat**

The nature of the threat may require immediate investigation if the caller claims  
Short time span to detonation (less than a hour)  
Specific descriptions of the devices  
Tone of the caller suggests valid threat and/or  
Political overtones to threat

Notify Security.

If local security is not available, call local police.

Evacuate the premises.

Post signs to advise people that the building is closed and return only after receiving clearance from emergency personnel.

## **Sit-Ins, Demonstrations and Riots**

Notify University Police. Security will maintain law and order, protect life and property, protect the rights of the University personnel, establish an effective control and will prevent confrontations.

Secure all entrances to the facility.

Sit-In Demonstrators shall not be allowed to disrupt normal activities.

Any arrest or citation will be as according to the University Police. Demonstrators will not be allowed on University areas.

## **Workplace Violence: See Hospital Policy 2.1.4 Violence in the Work Place**

## **APPENDIX 6**

### **Environmental Emergencies**

**Natural Disaster** (Floods, Earthquakes, and Hurricanes): If as a result of heavy rains or plumbing problems, the potential for flooding of a facility exists, area personnel should take the following measures to prevent the release of biological materials.

Inactivate all cultures that could possibly enter flood waters. Relocate all stock cultures to areas that are not at risk of flooding.

In the event of a "tornado watch," as announced by the National Weather Service, all active manipulation should cease and prepare for proper storage. Personnel should seek shelter away from windows (interior room) and at lower levels.

**Utility Emergencies** (Power outages): In most cases, containment of biological materials is not compromised during utility failures provided that facility personnel respond in an appropriate manner. In an electrical failure while using a biological safety cabinet.

Discontinue work with the biological material immediately.

Seal all cultures securely.

Decontaminate the work area with an appropriate disinfectant.

### **Refrigerator, Freezers, Walk-in, and Other Cold Storage Facilities**

This type of equipment must be labeled as to whom to contact person and emergency numbers in case of emergency, i.e., freezer breakdown.

All biological commodities must be inventoried on an annual basis. Expired and other unwanted material must be decontaminated properly. Materials for long term storage must be annually inspected and each container must be checked for cracks and other damages and properly disposed of replaced.

In the event of a freezer melt-down, all materials that are unable to be salvaged must be properly treated by autoclaving or properly disinfected before disposal.

## **APPENDIX 7**

### **Definition of Terms:**

**Laminar Flow:** Laminar flow is unidirectional air moving at a steady velocity along parallel lines. Laminar flow cabinets may or may not be biological safety cabinets.

**HEPA Filter:** High efficiency particulate air filter designed to remove particles, including microorganisms, from the air. HEPA filters are effective at trapping particulates and infectious agents, but not at capturing volatile chemicals or gas. Only certain classes of biological safety cabinets that are exhausted to the outside can be used when working with small amounts of volatile chemicals.

**Laminar Flow Clean Benches:** These **are not** biological safety cabinets and offer no worker or environmental protection. Clean benches must **never** be substituted for biological safety cabinets. Air is blown at the worker exposing them to whatever is present on the

bench. Clean benches should not be used for work involving cell cultures, toxins, volatile chemicals, infectious materials, or materials that may cause hypersensitivity to the worker - such as animal dander.

Laminar flow clean benches can have either horizontal or vertical airflow (see figure). HEPA-filtered air is discharged across the work surface to protect product on the bench from contamination. Vertical flow clean benches may have a sash similar to a biological safety cabinet but **air is discharged at the worker** under the sash. There is no air intake grill below the sash.

**Biological Safety Cabinet:** Biological safety cabinets are often referred to as "tissue culture hoods" or "laminar flow hoods".

**Biological safety cabinet** is the correct term. All biological safety cabinets use HEPA filters to treat exhaust air. Class II cabinets filter both exhaust and intake air to protect the worker and the environment from contamination as well as to protect product in the cabinet. See information below to learn more about the different classifications of biological safety cabinets and how to select the correct cabinet for your work.

**Chemical Fume Hoods:** Chemical fume hoods are used to protect workers from exposure to volatile chemicals. Neither the intake or exhaust air is HEPA filtered. Infectious materials should not be used in chemical fume hoods. These hoods are part of the facility and are tested annually by the Safety Office.

**Aerosols** A colloid of liquid or solid particles suspended in a gas usually air. There are several ways to create an aerosol, centrifuging, grinding, pouring, mixing, and pipetting to name a few. When there is a risk of an aerosol all work should be performed in a BSC.

**Laboratory centrifuge** An apparatus used in the laboratory for separating substances of different density or particle size, when suspended in a fluid, by spinning them about an axis in a suitable container.

**Rotor:** Primary component of a centrifuge which holds the material to be subjected to centrifugal force (in some form of tube/container) and which is rotated by the drive system.

## APPENDIX 8

### Types (Classes) of Biological Safety Cabinets

Nonvolatile chemicals can be used in all classes of biological safety cabinets. For volatile chemical use see table below. In certain cases, a charcoal filter may be added to prevent release of toxic chemicals into the atmosphere.

Type (Class)	Worker Protection	Product Protection	Environment Protection	Volatile Chemicals	Application
I	Yes	No	Yes	Yes*	Enclose equipment or procedures with a potential to generate aerosols (tissue homogenization, cage cleaning, etc).
II, A1 (was A)	Yes	Yes	Yes	No	Cell culture and infectious material procedures that do not include the use of volatile chemicals.
II, A2 (was B3)	Yes	Yes	Yes	Yes (minute amounts)**	Same as Type A but exhausted to outside. Minute quantities of hazardous chemicals may be used.
II, B1	Yes	Yes	Yes	Yes (minute amounts)**	Must be hard-ducted to exterior exhaust. Same procedures as Type II,A but manipulations of minute quantities of hazardous chemicals used with <i>in vitro</i> biological systems can be done.
II, B2	Yes	Yes	Yes	Yes (small amounts)	Cabinet has total-exhaust, no air is recirculated. This cabinet provides simultaneous primary biological and chemical containment. Care must be given as some chemicals can damage the filters or gaskets.

\* Special installation venting and/or filters may be needed. Air discharge into a room should not occur if volatile chemicals are to be used.

\*\* Chemical concentrations should not approach the lower explosion limits for the compound.

## APPENDIX 9

### Animal Biosafety Level 1

**Animal Biosafety Level 1** is suitable for work involving well characterized agents that are not known to cause disease in immunocompetent adult humans, and present minimal potential hazard to personnel and the environment.

ABSL-1 facilities should be separated from the general traffic patterns of the building and restricted as appropriate. Special containment equipment or facility design may be required as determined by appropriate risk assessment (See Section 2).

Personnel must have specific training in animal facility procedures and must be supervised by an individual with adequate knowledge of potential hazards and experimental animal procedures.

The following standard practices, safety equipment, and facility requirements apply to ABSL-1:

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

1. The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergency situations. Each institute must assure that worker safety and health concerns are addressed as part of the animal protocol review. Prior to beginning a study animal protocols must also be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC)<sup>5</sup> and the Institutional Biosafety Committee.

2. A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety professionals. The safety manual must be available and accessible. Personnel are advised of potential hazards and are required to read and follow instructions on practices and procedures.

3. Supervisor must ensure that animal care, laboratory and support personnel receive appropriate training regarding their duties, animal husbandry procedure, potential hazards, manipulations of infectious agents, necessary precautions to prevent hazard or exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates or additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.

4. Appropriate medical surveillance program is in place, as determined by risk assessment. The need for an animal allergy prevention program should be considered. Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care and manipulations.

Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women of child-bearing age should be provided information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

Personnel using respirators must be enrolled in an appropriately constituted respiratory protection program.

5. A sign incorporating safety information must be posted at the entrance to the areas where infectious materials and/or animals are housed or are manipulated. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of specific infectious agents is recommended when more than one agent is being used within an animal room. Security-sensitive agent information should be posted in accordance with the institutional policy.

Advance consideration should be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters

6. Access to the animal room is limited. Only those persons required for program or support purposes are authorized to enter the facility. All persons including facility personnel, service workers, and visitors are advised of the potential hazards (natural or research pathogens, allergens, etc) and are instructed on the appropriate safeguards.

7. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing. Gloves are worn to prevent skin contact with contaminated, infectious and hazardous materials, and when handling animals.

8. Gloves and personal protective equipment should be removed in a manner that minimizes transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated.

9. Persons must wash their hands after removing gloves, and before leaving the areas where infectious materials and/or animals are housed or are manipulated.
10. Eye and face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.
11. 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.
12. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
13. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.
14. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:
  - a. Needles and syringes or other sharp instruments are limited to use in the animal facility when there is no alternative for such procedures as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
  - b. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the work site as possible.
  - c. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
  - d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.
  - e. Equipment containing sharp edges and corners should be avoided.

15. Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent, and after any spills, splashes, or other overt contamination.
16. Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/or animals are housed or are manipulated.
17. An effective integrated pest management program is required.
18. All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof, covered containers for appropriate disposal in compliance with applicable institutional, local and state requirements. Decontaminate all potentially infectious materials before disposal using an effective method.

**B. *Special Practices***

None required.

**C. *Safety Equipment (Primary Barriers and Personal Protective Equipment)***

1. A risk assessment should determine the appropriate type of personal protective equipment to be utilized.
2. Special containment devices or equipment may be required as determined by appropriate risk assessment. Protective laboratory coats, gowns, or uniforms may be required to prevent contamination of personal clothing. Protective outer clothing is not worn outside areas where infectious materials and/or animals are housed or manipulated. Gowns and uniforms are not worn outside the facility.
3. Protective eyewear is worn when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates. Persons having contact with the NHP should assess risk of mucous membrane exposure and wear appropriate protective equipment (e.g., masks, goggles, faceshields, etc.) as needed.
4. Gloves are worn to protect hands from exposure to hazardous materials. A risk assessment should be performed to identify the appropriate glove for the task and alternatives to latex gloves should be available. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Gloves must not be worn

outside the animal rooms. Gloves and personal protective equipment should be removed in a manner that prohibits transfer of infectious materials. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste. Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Hand washing should occur after the removal of gloves.

**D. Laboratory Facilities (Secondary Barriers)**

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking. Access to the animal facility is restricted. Doors to areas where infectious materials and/or animals are housed, open inward, are self-closing, are kept closed when experimental animals are present, and should never be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.

2. The animal facility must have a sink for hand washing. Sink traps are filled with water, and/or appropriate liquid to prevent the migration of vermin and gases.

3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant. It is recommended that penetrations in floors, walls and ceiling surfaces are sealed, to include openings around ducts, doors and door frames, to facilitate pest control and proper cleaning. Floors must be slip resistant, impervious to liquids, and resistant to chemicals.

4. Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning. Chairs used in animal area must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Sharp edges and corners should be avoided.

5. External windows are not recommended; if present windows must be resistant to breakage. Where possible, windows should be sealed. If the animal facility has windows that open, they are fitted with fly screens. The presence of windows may impact facility security and therefore should be assessed by security personnel.

6. Ventilation should be provided in accordance with the *Guide for Care and Use of Laboratory Animals*.<sup>1</sup> No recirculation of exhaust air

should occur. It is recommended that animal rooms have inward directional airflow. Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.

7. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.

8. If floor drains are provided, the traps are filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.

9. Cages are washed manually or preferably in a mechanical cage washer. The mechanical cage washer should have a final rinse temperature of at least 180°F.

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

11. Emergency eyewash and shower are readily available; location is determined by risk assessment.

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## **Animal Biosafety Level 2**

**Animal Biosafety Level 2** builds upon the practices, procedures, containment equipment, and facility requirements of ABSL-1. ABSL-2 is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment. It also addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure.

ABSL-2 requires that 1) access to the animal facility is restricted; 2) personnel must have specific training in animal facility procedures, the handling of infected animals and the manipulation of pathogenic agents; 3) personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations and husbandry procedures; and 4) procedures involving the manipulation of infectious materials, or where aerosols or splashes may be created, should be conducted in BSCs or by use of other physical containment equipment.

Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment. Implementation of employee occupational health programs should be considered. The following standard and special practices, safety equipment, and facility requirements apply to ABSL-2:

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

1. The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergency situations.

## **Animal Biosafety Level 2**

Each institute must assure that worker safety and health concerns are addressed as part of the animal protocol review. Prior to beginning a study animal protocols must also be reviewed and approved by the IACUC5 and the Institutional Biosafety Committee.

2. A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety professionals.

The safety manual must be available and accessible. Personnel are advised of potential hazards, and are required to read and follow instructions on practices and procedures.

Consideration should be given to specific biohazards unique to the animal species and protocol in use.

3. Supervisor must ensure that animal care, laboratory and support personnel receive appropriate training regarding their duties, animal

husbandry procedure, potential hazards, manipulations of infectious agents, necessary precautions to prevent hazard or exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates or additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.

4. Appropriate medical surveillance program is in place, as determined by risk assessment. The need for an animal allergy prevention program should be considered. Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care and manipulations. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women of child-bearing age should be provided information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance. Personnel using respirators must be enrolled in an appropriately constituted respiratory protection program.

5. A sign incorporating the universal biohazard symbol must be posted at the entrance to areas where infectious materials and/or animals are housed or are manipulated when infectious agents are present. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of specific infectious agents is recommended when more than one agent is being used within an animal room. Security-sensitive agent information and occupational health requirements should be posted in accordance with the institutional policy. Advance consideration should be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters. 1,3,4

6. Access to the animal room is limited. Only those persons required for program or support purposes are authorized to enter the animal facility and the areas where infectious materials and/or animals are housed or are manipulated. All persons including facility personnel, service workers, and visitors are advised of the potential hazards

(natural or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.

7. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing. Gloves are worn to prevent skin contact with contaminated, infectious and hazardous materials and when handling animals. Gloves and personal protective equipment should be removed in a manner that minimizes transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated. Persons must wash their hands after removing gloves, and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Eye and face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.

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

9. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.

10. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.

11. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:

- a. Needles and syringes or other sharp instruments are limited to use in the animal facility when there is no alternative for such procedures as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
- b. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the work site as possible.
- c. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

- d. Broken glassware must not be handled directly; it should be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.
  - e. Equipment containing sharp edges and corners should be avoided.
12. Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent, and after any spills, splashes, or other overt contamination.
13. Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/or animals are housed or are manipulated.
14. An effective integrated pest management program is required
15. All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof containers for appropriate disposal in compliance with applicable institutional, local and state requirements. Decontaminate of all potentially infectious materials before disposal using an effective method.

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

1. Animal care staff, laboratory and routine support personnel must be provided a medical surveillance program as dictated by the risk assessment, and administered appropriate immunizations for agents handled or potentially present, before entry into animal rooms. When appropriate, a baseline serum sample should be stored.
2. Procedures involving a high potential for generating aerosols should be conducted within a biosafety cabinet or other physical containment device. When a procedure cannot be performed within a biosafety cabinet, a combination of personal protective equipment and other containment devices must be used. Consideration should be given to the use of restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint devices, chemical restraint medications, etc).
3. Decontamination is recommended for all potentially infectious materials and animal waste before movement outside the areas where infectious materials and/or animals are housed or are manipulated by an appropriate method (e.g. autoclave, chemical disinfection, or other approved decontamination methods). This includes potentially

infectious animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse. Consideration should be given to means for decontaminating routine husbandry equipment, sensitive electronic and medical equipment. Materials to be decontaminated outside of the immediate areas where infectious materials and/or animals are housed or are manipulated must be placed in a durable, leak proof, covered container and secured for transport. The outer surface of the container is disinfected prior to moving materials. The transport container must contain a universal biohazard label. Develop and implement an appropriate waste disposal program in compliance with applicable institutional, local and state requirements. Autoclaving of content prior to incineration is recommended.

4. Equipment, cages, and racks should be handled in manner that minimizes contamination of other areas. Equipment must be decontaminated before repair, maintenance, or removal from the areas where infectious materials and/or animals are housed or are manipulated. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.

5. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the safety manual. All such incidents must be reported to the animal facility supervisor or personnel designated by the institution. Medical evaluation, surveillance, and treatment should be provided as appropriate and records maintained.

### **C. *Safety Equipment (Primary Barriers and Personal Protective Equipment)***

1. Properly maintained BSCs, personal protective equipment (e.g., gloves, lab coats, face shields, respirators, etc.) and/or other physical containment devices or equipment, are used whenever conducting procedures with a potential for creating aerosols or splashes. These include necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals. When indicated by risk assessment, animals are housed in primary biosafety containment equipment appropriate for the animal species, such as solid wall and bottom cages covered with filter bonnets for rodents, or larger cages placed in inward flow ventilated enclosures or other equivalent primary containment systems for larger animal cages.

2. A risk assessment should determine the appropriate type of personal protective equipment to be utilized.

Scrub suits and uniforms are removed before leaving the animal facility. Reusable clothing is appropriately contained and decontaminated before being laundered. Laboratory and protective clothing should never be taken home. Gowns, uniforms, laboratory coats and personal protective equipment are worn while in the areas where infectious materials and/or animals are housed or manipulated and removed prior to exiting. Disposable personal protective equipment and other contaminated waste are appropriately contained and decontaminated prior to disposal.

3. Eye and face protection (mask, goggles, face shield or other splatter guard) are used for anticipated splashes/ sprays from infectious or other hazardous materials and when the animal or microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates. Persons having contact with the NHP should assess risk of mucous membrane exposure and wear appropriate protective equipment (e.g., masks, goggles, faceshields, etc.) as needed. Respiratory protection is worn based upon risk assessment.

4. Gloves are worn to protect hands from exposure to hazardous materials. A risk assessment should be performed to identify the appropriate glove for the task and alternatives to latex gloves should be available. Gloves are changed when contaminated, integrity has been compromised, or when otherwise necessary. Gloves must not be worn outside the animal rooms. Gloves and personal protective equipment should be removed in a manner that prohibits transfer of infectious materials. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste. Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Hand washing should occur after the removal of gloves.

#### ***D. Laboratory Facilities (Secondary Barriers)***

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking. Access to the animal facility is restricted. Doors to areas where infectious materials and/or animals are housed, open inward, are self-closing, are kept closed when experimental animals are present, and should never be propped open.

Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.

2. A hand washing sink is located at the exit of the areas where infectious materials and/or animals are housed or are manipulated. Additional sinks for hand washing should be located in other appropriate locations within the facility. If the animal facility has segregated areas where infectious materials and/or animals are housed or manipulated, a sink must also be available for hand washing at the exit from each segregated area. Sink traps are filled with water, and/or appropriate liquid to prevent the migration of vermin and gases.

3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant. Penetrations in floors, walls and ceiling surfaces are sealed, to include openings around ducts, doors and door frames, to facilitate pest control and proper cleaning. Floors must be slip resistant, impervious to liquids, and resistant to chemicals.

4. Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning. Furniture should be minimized. Chairs used in animal area must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Sharp edges and corners should be avoided.

5. External windows are not recommended; if present, windows should be sealed and must be resistant to breakage. The presence of windows may impact facility security and therefore should be assessed by security personnel.

6. Ventilation should be provided in accordance with the *Guide for Care and Use of Laboratory Animals*.<sup>1</sup> The direction of airflow into the animal facility is inward; animal rooms should maintain inward directional airflow compared to adjoining hallways. A ducted exhaust air ventilation system is provided. Exhaust air is discharged to the outside without being recirculated to other rooms. Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.

7. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas, to facilitate cleaning and minimize the accumulation of debris or fomites.
8. Floor drains must be maintained and filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.
9. Cages should be autoclaved or otherwise decontaminated prior to washing. Mechanical cage washer should have a final rinse temperature of at least 180°F. The cage wash area should be designed to accommodate the use of high pressure spray systems, humidity, strong chemical disinfectants and 180°F water temperatures, during the cage/equipment cleaning process.
10. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
11. If BSCs are present, they must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. Correct performance of the BSCs should be recertified at least once a year. All BSCs should be used according to manufacturer's recommendation, to protect the worker and avoid creating a hazardous environment from volatile chemical and gases.
12. If vacuum service (i.e., central or local) is provided, each service connection should be fitted with liquid disinfectant traps and an in-line HEPA filter, placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement.
13. An autoclave should be considered in the animal facility to facilitate decontamination of infectious materials and waste.
14. Emergency eyewash and shower are readily available; location is determined by risk assessment.

## **Animal Biosafety Level 3**

**Animal Biosafety Level 3** involves practices suitable for work with laboratory animals infected with indigenous or exotic agents, agents that present a potential for aerosol transmission and agents causing serious or potentially lethal disease. ABSL-3 builds upon the standard practices, procedures, containment equipment, and facility requirements of ABSL-2. ABSL-3 laboratory has special engineering and design features. ABSL-3 requires that 1) access to the animal facility is restricted; 2) personnel must have specific training in animal facility procedures, the handling of infected animals and the manipulation of potentially lethal agents; 3) personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations and husbandry procedures; and 4) procedures involving the manipulation of infectious materials, or where aerosols or splashes may be created, must be conducted in BSCs or by use of other physical containment equipment. Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment. Employee occupational health programs must be implemented. The following standard and special safety practices, safety equipment, and facility requirements apply to ABSL-3

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

1. The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergency situations. Each institute must assure that worker safety and health concerns are addressed as part of the animal protocol review. Prior to beginning a study animal protocols must also be reviewed and approved by the IACUC5 and the Institutional Biosafety Committee.

2. A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety professionals. The safety manual must be available and accessible. Personnel are advised of potential and special hazards, and are required to read and follow instructions on practices and procedures. Consideration should be given to specific biohazards unique to the animal species and protocol in use.

3. Supervisor must ensure that animal care, laboratory and support personnel receive appropriate training regarding their duties, animal husbandry procedure, potential hazards, manipulations of infectious

agents, necessary precautions to prevent hazard or exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates or additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.

4. Appropriate medical surveillance program is in place, as determined by risk assessment. The need for an animal allergy prevention program should be considered. Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care and manipulations. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women of child-bearing age should be provided information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance. Personnel using respirators must be enrolled in an appropriately constituted respiratory protection program.

5. A sign incorporating the universal biohazard symbol must be posted at the entrance to areas where infectious materials and/or animals are housed or are manipulated. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of specific infectious agents is recommended when more than one agent is being used within an animal room. Security-sensitive agent information and occupational health requirements should be posted in accordance with the institutional policy. Advance consideration should be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.

6. Access to the animal room is limited to the fewest number of individuals possible. Only those persons required for program or support purposes are authorized to enter the animal facility and the areas where infectious materials and/or animals are housed or are manipulated. All persons including facility personnel, service workers, and visitors are advised of the potential hazards (natural or research pathogens, allergens, etc) and are instructed on the appropriate safeguards.

7. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing. Gloves are worn to prevent skin contact with contaminated, infectious/ and hazardous materials and when handling animals. Double-glove practices should be used when dictated by risk assessment. Gloves and personal protective equipment should be removed in a manner that minimizes transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated. Persons must wash their hands after removing gloves, and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Eye and face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.

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

9. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.

10. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.

11. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:

- a. Needles and syringes or other sharp instruments are limited to use in the animal facility when there is no alternative for such procedures as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
- b. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the work site as possible.
- c. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

- d. Broken glassware must not be handled directly; it should be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.
  - e. Equipment containing sharp edges and corners should be avoided.
12. Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent, and after any spills, splashes, or other overt contamination.
13. Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/or animals are housed or are manipulated.
14. An effective integrated pest management program is required.
15. All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof containers for appropriate disposal in compliance with applicable institutional, local and state requirements. Decontaminate of all potentially infectious materials before disposal using an effective method.

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

1. Animal care staff, laboratory and routine support personnel must be provided a medical surveillance program as dictated by the risk assessment, and administered appropriate immunizations for agents handled or potentially present, before entry into animal rooms. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.
2. All procedures involving the manipulation of infectious materials, handling infected animals or the generations of aerosols must be conducted within BSCs or other physical containment devices when practical. When a procedure cannot be performed within a biosafety cabinet, a combination of personal protective equipment and other containment devices must be used. Consideration should be given to the use of restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint devices, chemical restraint medications, etc).
3. The risk of infectious aerosols from infected animals or their bedding also can be reduced if animals are housed in containment caging systems (such as solid wall and bottom cages covered with

filter bonnets, open cages placed in inward flow ventilated enclosures, HEPA-filter isolators and caging systems, or other equivalent primary containment systems).

4. Actively ventilated caging systems must be designed to prevent the escape of microorganisms from the cage. Exhaust plenums for these systems should be sealed to prevent escape of microorganisms if the ventilation system becomes static, and the exhaust must be HEPA filtered. Safety mechanisms should be in place that prevent the cages and exhaust plenums from becoming positive to the surrounding area should the exhaust fan fail. The system should also be alarmed to indicate when operational malfunctions occur.

5. A method for decontaminating all infectious materials must be available within the facility, preferably within the areas where infectious materials and/or animals are housed or are manipulated (e.g. autoclave, chemical disinfection, or other approved decontamination methods). Consideration should be given to means for decontaminating routine husbandry equipment, sensitive electronic and medical equipment. Decontaminate all potential infectious materials (including animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse) before removal from the areas where infectious materials and/or animals are housed or are manipulated by an appropriate method. It is recommended that animal bedding and waste be decontaminated prior to manipulation and before removal from the areas where infectious materials and/or animals are housed or are manipulated, preferably within the caging system. Develop and implement an appropriate waste disposal program in compliance with applicable institutional, local and state requirements. Autoclaving of content prior to incineration is recommended.

6. Equipment, cages, and racks should be handled in manner that minimizes contamination of other areas. Equipment must be decontaminated before repair, maintenance, or removal from the areas where infectious materials and/or animals are housed or are manipulated. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.

7. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the safety manual. All such incidents must be reported to the animal facility supervisor or personnel designated by the institution. Medical

evaluation, surveillance, and treatment should be provided as appropriate and records maintained.

### **C. Safety Equipment (Primary Barriers and Personal Protective Equipment)**

1. Properly maintained BSCs, and other physical containment devices or equipment, should be used for all manipulations for infectious materials and when possible, animals. These manipulations include necropsy, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals. The risk of infectious aerosols from infected animals or bedding can be reduced through the use of primary barrier systems. These systems may include solid wall and bottom cages covered with filter bonnets; ventilated cage rack systems; or for larger cages placed in inward flow ventilated enclosures or other equivalent systems or devices.

2. A risk assessment should determine the appropriate type of personal protective equipment to be utilized. Protective clothing such as uniforms or scrub suits is worn by personnel within the animal facility. Reusable clothing is appropriately contained and decontaminated before being laundered. Laboratory and protective clothing should never be taken home. Disposable personal protective equipment such as non-woven olefin cover-all suits, wrap-around or solid-front gowns should be worn over this clothing, before entering the areas where infectious materials and/or animals are housed or manipulated. Front-button laboratory coats are unsuitable. Disposable personal protective equipment must be removed when leaving the areas where infectious materials and/or animals are housed or are manipulated. Scrub suits and uniforms are removed before leaving the animal facility. Disposable personal protective equipment and other contaminated waste are appropriately contained and decontaminated prior to disposal.

3. Appropriate eye, face and respiratory protection are worn by all personnel entering areas where infectious materials and/or animals are housed or are manipulated. To prevent cross contamination boots, shoe covers, or other protective footwear, are used where indicated. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates.

4. Gloves are worn to protect hands from exposure to hazardous materials. A risk assessment should be performed to identify the appropriate glove for the task and alternatives to latex gloves should

be available. Procedures may require the use of wearing two pairs of gloves (double-glove). Gloves are changed when contaminated, integrity has been compromised, or when otherwise necessary. Gloves must not be worn outside the animal rooms. Gloves and personal protective equipment should be removed in a manner that prohibits transfer of infectious materials. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste. Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Hand washing should occur after the removal of gloves.

#### ***D. Laboratory Facilities (Secondary Barriers)***

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking. Access to the animal facility is restricted. Doors to areas where infectious materials and/or animals are housed, open inward, are self-closing, are kept closed when experimental animals are present, and should never be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically. Entry into the containment area is via a double-door entry which constitutes an anteroom/airlock and a change room. Showers may be considered based on risk assessment. An additional double-door access anteroom or double-doored autoclave may be provided for movement of supplies and wastes into and out of the facility.

2. A hand washing sink is located at the exit of the areas where infectious materials and/or animals are housed or are manipulated. Additional sinks for hand washing should be located in other appropriate locations within the facility. The sink should be hands-free or automatically operated. If the animal facility has multiple segregated areas where infectious materials and/or animals are housed or are manipulated, a sink must also be available for hand washing at the exit from each segregated area. Sink traps are filled with water, and/or appropriate liquid to prevent the migration of vermin and gases.

3. The animal facility is designed, constructed, and maintained to facilitate cleaning, decontamination and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant. Penetrations in floors, walls and ceiling surfaces are sealed, to include openings around ducts, doors and door frames, to facilitate pest control, proper cleaning and decontamination. Walls, floors and ceilings should form a sealed and sanitizable surface. Floors must be slip resistant,

impervious to liquids, and resistant to chemicals. Flooring is seamless, sealed resilient or poured floors, with integral cove bases. Decontamination of an entire animal room should be considered when there has been gross contamination of the space, significant changes in usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the animal room must be based on the risk assessment.

4. Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning. Furniture should be minimized. Chairs used in animal area must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Sharp edges and corners should be avoided.

5. External windows are not recommended; if present, all windows must be sealed and must be resistant to breakage. The presence of windows may impact facility security and therefore should be assessed by security personnel.

6. Ventilation to the facility should be provided in accordance with the *Guide for Care and Use of Laboratory Animals*.<sup>1</sup> The direction of airflow into the animal facility is inward; animal rooms should maintain inward directional airflow compared to adjoining hallways. A ducted exhaust air ventilation system is provided. Exhaust air is discharged to the outside without being recirculated to other rooms.

This system creates directional airflow which draws air into the animal room from "clean" areas and toward "contaminated" areas.

Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process. Filtration and other treatments of the exhaust air may not be required, but should be considered based on site requirements, specific agent manipulations and use conditions. The exhaust must be dispersed away from occupied areas and air intakes, or the exhaust must be HEPA-filtered. Personnel must verify that the direction of the airflow (into the animal areas) is proper. It is recommended that a visual monitoring device that indicates directional inward airflow be provided at the animal room entry. The ABSL-3 animal facility shall be designed such that under failure conditions the airflow will not be reversed. Audible alarms should be considered to notify personnel of ventilation and HVAC system failure.

7. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas, to facilitate cleaning and minimize the accumulation of debris or fomites.

8. Floor drains must be maintained and filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.

9. Cages are washed in a mechanical cage washer. The mechanical cage washer has a final rinse temperature of at least 180°F. Cages should be autoclaved or otherwise decontaminated prior to removal from ABSL-3 space. The cage wash facility should be designed and constructed to accommodate high pressure spray systems, humidity, strong chemical disinfectants and 180°F water temperatures, during the cage cleaning process.

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

11. BSCs (Class II, Class III) must be installed so that fluctuations of the room air supply and exhaust do not interfere with its proper operations. Class II BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be certified at least annually to assure correct performance. Class III BSCs must supply air in such a manner that prevents positive pressurization of the cabinet or the laboratory room. All BSCs should be used according to manufacturers' recommendations. When applicable, equipment that may produce infectious aerosols must be contained in devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the animal facility. These HEPA filters should be tested and/or replaced at least annually.

12. An autoclave is available which is convenient to the animal rooms where the biohazard is contained. The autoclave is utilized to decontaminate infectious materials and waste before moving it to the other areas of the facility. If not convenient to areas where infectious materials and/or animals are housed or are manipulated, special

practices should be developed for transport of infectious materials designated alternate location/s within the facility.

13. Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.

14. Emergency eyewash and shower are readily available; location is determined by risk assessment.

15. The ABSL-3 facility design and operational procedures must be documented. The facility must be tested to verify that the design and operational parameters have been met prior to use. Facilities should be re-verified at least annually against these procedures as modified by operational experience.

16. Additional environmental protection (e.g., personnel showers, HEPA filtration of exhaust air, containment of other piped services, and the provision of effluent decontamination) should be considered if recommended by the agent summary statement, as determined by risk assessment of the site conditions, or other applicable federal, state or local regulations.

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## APPENDIX 10

### Tips to prevent contamination:

Clean water baths frequently and/or treat water in bath.

Clean the inside of incubators frequently, particularly the water tray. Use sterile water in the bottom of the incubator or water tray.

Use HEPA filters on incubator CO<sub>2</sub> and air intake lines. Replace regularly.

Lab coat sleeves can introduce contaminants to biological safety cabinets and incubators. Use coats designated for working in the biological safety cabinet or tissue culture area, launder frequently. Use disposable sleeve guards if contamination has been a problem.

Never pour media, remove with vacuum, and replace with disposal pipettes.

Do not leave flasks of waste media in cabinet, clean after every use.

On a regular basis, decontaminate under the air grilles and wherever parts are removable. Media is commonly splattered on the front grille allowing fungus to grow undetected on the under surface of the grille.

Decontaminate the surface of carts or trays used to transfer culture flasks between the incubator and the biological safety cabinet or microscope.

Keep pipette aids cleaned, especially the nosepiece, and replace filters regularly.

Clean and disinfect vacuum tubing.

Keep the water in the incubator's water jacket full. If water levels in the jacket drop, the ceiling of the incubator will be cooler causing condensate to form. Water then drops onto shelves and cell culture containers.

Check port plugs and septums for contamination in incubator interior; they may trap moisture and harbor fungi.

Most contamination problems can be traced to incubators, water baths, or using poor aseptic technique. Don't be lulled into believing that the use of UV lights or flaming containers will keep you contamination free.

## APPENDIX 11

### USEFUL WEBSITES

<http://www.cdc.gov/>

***NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)***, current edition;

<http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html>

***Biosafety in Microbiological and Biomedical Laboratories (BMBL)***, current edition.

<http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm>

**Material Safety Data Sheets (MSDS)**, for personnel working in the life sciences as quick safety reference material relating to infectious micro-organisms.

<http://www.phac-aspc.gc.ca/msds-ftss/index.html>

Information about biological safety cabinets

<http://www.niehs.nih.gov/odhsb/biosafe/bsc/bsc.htm>

Information for Natural Disasters & Severe Weather

<http://www.bt.cdc.gov/disasters/index.asp>

## APPENDIX 12

Link to the most current NIH Guidelines for:

### **Research Involving Recombinant or Synthetic Nucleic Acid Molecules**

[http://oba.od.nih.gov/rdna/nih\\_guidelines\\_oba.html](http://oba.od.nih.gov/rdna/nih_guidelines_oba.html)

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## APPENDIX 13

Date: \_\_\_\_\_

Your compliance rate for \_\_\_\_\_ for \_\_\_\_\_ was \_\_\_\_\_% and your conversion  
(Department) (Year)  
rate was \_\_\_\_%. You are in the \_\_\_\_\_ risk category.

\_\_\_\_\_ is your assigned month for testing.

(Month)

**Please send an updated list of your employees' names and social security numbers by email or fax (675-4647) upon receipt of this memo.**

Please indicate your employee's classification (e.g. M.D., PhD, R.N., Adm. Secretary, Clerk, etc)

*Your employees will be given a pink copy of their TB skin test/evaluation upon completion of their testing/evaluation with instructions to give this to their supervisor or the supervisor's designee. Employees of a department e.g. Surgery Department, will be instructed to give the pink copy to the departmental secretary. This will be the secretary in their department who makes the arrangements for the TB testing and evaluation for the department.*

**It is imperative that the person designated to collect the pink sheets have a copy of the employee list that has been sent to the Occupational Health Clinic for TB skin testing/evaluation and that they check the names off the list as the employees turn in their pink copies. This is the only way each unit or department will know who has not completed testing/evaluation by the end of the assigned month and give them the opportunity to bring any non compliant employee into compliance in a timely manner.**

Per the Tuberculosis Control Resolutions amended and passed by the Clinical Board 11/20/00 all employees in LSUHSC hospital and its outpatient clinics; direct patient contact or not, paid or volunteer; fall under the scope of tuberculosis surveillance and must have TB testing/evaluation a minimum of annually and more frequently if their risk factors and exposure incidences so indicate. For those employees who persist in remaining non compliant for TB testing/evaluation following the exhaustion of the steps delineated in the TB Control Resolutions patient care privileges will be suspended until the required screening is completed.

**(This also applies to all research/laboratory personnel)**

Thank you for your assistance and cooperation in taking care of this mandatory requirement..

**References:**

NIH Guidelines current edition

BMBL current edition

Applied Biosafety Journal of the American Biological Safety Association

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