



# **Biosafety Manual**

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## **1.0 INTRODUCTION**

### **1.1 PURPOSE**

The purpose of the FIU Biosafety manual is to minimize employee and student exposure to biohazardous agents and to ensure that all university laboratories operate in compliance with regulations mandated by governmental regulatory and credentialing agencies.

### **1.2 SCOPE**

The procedures set forth in the FIU Biosafety manual apply to all university laboratory facilities in which exposure to known or potentially biohazardous agents may occur.

### **1.3 PROGRAM ADMINISTRATION**

#### **Director of Environmental Health and Safety (EH&S)**

The Director of Environmental Health and Safety is responsible for the following:

- Manages health and safety programs that provide for University wide compliance with the requirements for laboratory safety
- Administers resources as assigned in order to meet this objective.
- Recommends to university officials those policies and procedures to be implemented in order to provide for a safe work environment in teaching and research laboratories

#### **Biosafety Officer**

The Biosafety Officer (BSO) shall assist Department Heads, Principal Investigators and Laboratory Managers in achieving compliance with laboratory biohazard safety standards and the requirements of the Biosafety Manual by doing the following:

- Assisting in the selection of best laboratory safety practices, personal protective equipment, and engineering controls
- Conducting laboratory safety inspections, at the frequency prescribed by the 'degree of hazard' of each laboratory
- Investigating all reported accidents that result in personnel or environmental exposure to hazardous materials and recommending corrective action to reduce the potential for recurrence
- Assuring the adequacy of clean-up and decontamination procedures in situations where accidents have resulted in contamination of laboratory areas
- Facilitating and scheduling appropriate training and dissemination of information in order to promote safe laboratory practices
- Monitoring laboratory personnel for potential exposure to hazardous substances
- Providing guidance on administrative and procedural controls for the safe management of regulated substances

- Facilitating safe storage, handling and ultimate disposal of biohazardous wastes generated by laboratories
- Maintaining the Manual to address changes in regulations, technology, etc
- Assisting the IBC and IACUC in protocol review and other compliance activities.

### **Institutional Biosafety Committee**

The Institutional Biosafety Committee (IBC) is advisory to the university on all matters regarding the safe use of recombinant DNA. It is the IBC's responsibility to establish and monitor policies and procedures that meet or exceed applicable regulations. All institutions awarded NIH funding are required to form IBCs which function per the NIH Institutional Biosafety Committee Guidelines. Critical duties of the IBC include:

- Reviewing recombinant DNA research conducted at or sponsored by the institution for compliance with the *NIH Guidelines* and approving those research projects that are found to conform with the *NIH Guidelines*.
- Notifying the Principal Investigator of the results of the Institutional Biosafety Committee's review and approval.
- Lowering and setting containment levels for certain experiments as specified by NIH guidelines.
- Periodically reviewing recombinant DNA research conducted at the institution to ensure compliance with the *NIH Guidelines*.
- Adopting emergency plans covering accidental spills and personnel contamination resulting from recombinant DNA research.

### **Institutional Animal Care and Use Committee**

The Animal Welfare Act requires that each research facility establish an Institutional Animal Care and Use Committee (IACUC) to oversee the humane care and use of regulated animals. It is important that the IACUC consider the general public's concerns regarding the welfare of animals used in research, as well as the needs of the facility. Responsibilities of the IACUC include:

- Reviewing protocols for all research projects involving animal subjects to ensure ethical and humane treatment of animals and compliance with applicable regulations
- Requiring approval of protocols prior to initiating new research or altering the protocol of existing research protocols involving test animals.
- Requiring consideration of alternative methods to replace the use of animals; reduce the number of animals needed; or refine the procedure to minimize discomfort, pain, and distress to the research animals.
- Ensuring activities do not duplicate previous experiments.
- Ensuring researchers provide sufficient documentation to make this determination by providing information such as the dates and databases covered by the literature search, and any other sources considered.

### **Laboratory Manager/Principal Investigator/Instructor**

The Principal Investigator, Instructor or Laboratory Manager shall serve as the supervisor of the laboratory and shall be responsible and accountable for the following:

- Ensuring that laboratory personnel and students are advised of, and follow prudent safety practices; that protective equipment is available and in working order; and that the specific precautions applicable to the type of work being conducted has been provided
- Assuring that emergency response procedures for the area(s) under their control are maintained current and appropriate for the type occurrences to be expected in such locations
- Conducting periodic laboratory safety and housekeeping inspections in order to maintain the laboratory in a safe working condition
- Assuring access control procedures have been developed and are complied with for the laboratory or work area under their control
- Maintaining a basic understanding of the current legal requirements applicable to the use of regulated/hazardous/biohazardous substances in the laboratory
- Identifying and assuring the availability of appropriate personal protective equipment
- Assuring that laboratory conditions and equipment are appropriate for the type of work planned
- Identifying and reporting maintenance and repair requirements to the Facilities Management/Maintenance department
- Providing timely notification to the Department of Environmental Health & Safety of process, procedural or facility related changes within their area of operation which are likely to change the hazard rating assigned to that location
- Identifying those substances and equipment, such as explosive materials or lasers, used in their laboratories which may pose a high risk of injury and/or property damage and implementing procedures to control exposures
- Complying with appropriate approval procedures, when required, for conducting high risk research activities
- Arranging for immediate medical attention and reporting of any incident that results in:
  - Injury
  - Exposure to biohazards at or above established thresholds
- Assisting the Department of Environmental Health & Safety in investigating occurrences that result in exposures, injuries and/or property damage
- Notifying the Department of Environmental Health & Safety of problems related to the general operation and implementation of laboratory safety practices and engineering controls

### **Employees & Students**

Each individual is responsible for planning and conducting laboratory activities in accordance with instructions received from instructors, laboratory managers, and principal investigators; and in accordance with appropriate laboratory safety guidelines. Students and laboratory workers shall comply with the following minimum guidelines:

- Knowing and complying with the safety guidelines, regulations and procedures required for tasks assigned
- Reporting unsafe conditions to the principal investigator, immediate supervisor or the Department of Environmental Health & Safety
- Reporting to the principal investigator or immediate supervisor facts pertaining to any accident resulting in exposures, injury and/or property damage
- Following instructions as given

### **Contractors**

Contractors performing work on FIU premises are responsible for conducting their activities in accordance with established environmental, health and safety procedures prescribed by their organization's health and safety plan, or as otherwise instructed by the FIU Facilities Management project managers.

*Note: The Biosafety Officer, principal investigator and laboratory manager may make specific recommendations to the contractor through the Facilities Management project manager regarding work to be carried out by contractors in University laboratories.*

### **Visitors**

Visitors to laboratories on FIU campuses are required to abide by the appropriate safety recommendations included in this Manual. However, it is the specific responsibility of staff and laboratory personnel to advise visitors of potential exposures and to assure they are appropriately protected prior to accessing locations where such exposures are likely.

## **2.0 PRINCIPLES OF BIOSAFETY**

### **2.1 CONTAINMENT**

Containment describes methods for managing infectious materials in the laboratory environment where they are being handled or maintained. By adhering to proper containment practices and procedures, the exposure to laboratory workers, outside persons, and the environment, is limited.

Primary containment: Good microbiological techniques, as well as proper use of safety equipment, provide for the protection of personnel and the immediate laboratory environment from exposure to infectious agents. Examples of primary containment include personal protective equipment (gloves, goggles, lab coats) and biological safety cabinets. The biological safety cabinet is the foremost primary containment device as it offers a variety of protective measures for the individuals using them.

Secondary containment: A combination of facility design and operational practices helps to protect the outside environment from exposure to potentially infectious agents.

### **2.2 BIOHAZARDS**

Biohazardous materials are infectious agents or biologically-derived infectious materials that present a risk to the health of humans and animals. Exposure may occur directly through infection or through some type of damage to the environment. The following are some examples of biohazardous materials:

- Human, animal, and plant pathogens which include bacteria, plasmids, prions, rickettsia, fungi, viruses and parasites
- All human blood and blood products
- All human tissue and body fluids which may be contaminated
- Culture cells, which include all human and some animal, and whatever type of potentially infectious agents that these cells may contain
- Toxins
- Certain recombinant-DNA products
- Clinical specimens
- Any animals which have been inoculated with a potentially infected material as well as animal tissues, animal bedding and animal waste materials.

### **2.3 SAFE LABORATORY PRACTICES AND TECHNIQUES**

The most important element of containment is strict adherence to standard microbiological practices and techniques. Following basic microbiological techniques is of utmost importance in containment within the laboratory environment. Laboratory personnel working with infectious agents or potentially infected materials must be aware of potential hazards, and must be trained and proficient in the practices and techniques



required to handle such material safely. Within the University, each supervisor is responsible for providing or arranging the appropriate training of personnel. The FIU Department of Environmental Health and Safety provides training for personnel in conjunction with the supervisor.

Laboratory personnel shall be advised of special hazards and shall be required to read and follow the required practices and procedures. Principal Investigators (PI) working at FIU should be trained and knowledgeable in appropriate laboratory techniques, safety procedures, and hazards of agents that they work with and they must be responsible for the conduct of work with any infectious agents or material. If needed, this individual shall consult with the Biosafety Officer or other health and safety professionals with regard to risk assessment.

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

There may be occasions when the training and experience of laboratory personnel, the safety practices they employ, and techniques used must be supplemented by appropriate facility design and engineering features, safety equipment, and management practices.

### 2.4 BIOSAFETY: BASIC PRACTICES

The following is a list of basic proper biosafety techniques/guidelines to follow when conducting experiments at FIU:

- Protective clothing, such as lab coats, must be worn during all work and research activities conducted in the laboratory and in accordance with the type of work being performed. **No open-toed shoes are allowed in the laboratory.** Shoes should be appropriate for work involved and should have non-slip soles
- Gloves must be worn at all times, particularly when working with infectious materials. These should be decontaminated with other laboratory waste before disposal
- Remove gloves by the inside-out technique: Using one gloved hand, pinch the cuff of the other glove and pull it from the inside out and off the hand. Then, using your index finger, dig into the cuff of the other glove and remove it using the inside out method as well.
- Hands should be washed before leaving the laboratory area.
- All contaminated or infectious solid materials must be properly disposed of in biohazard bags and then autoclaved before they are removed from the campus.

- Eye goggles and face masks should be worn when working with infectious materials in the event that splashes or aerosols are created during routine work conditions.
- **Eating, drinking, smoking, and storing of food in the laboratory is prohibited**
- All work areas should be clean and uncluttered.
- Creation of aerosols should be minimized during technical procedures.
- When working with blood or blood products, extra care should be taken and appropriate Personal Protective Equipment must be worn.
- When handling needles or syringes, appropriate procedures must be followed.  
**DO NOT recap bent or broken needles**
- Use appropriate sharps containers for needles/syringes, broken glass, etc.
- Only authorized personnel should be allowed in the work areas(s).
- Keep laboratory doors closed while working with infectious materials.
- Workers should decontaminate work area immediately following completion of work or after a biohazardous spill.
- Personnel should not be working alone when handling infectious materials or other hazardous substances.

The ultimate responsibility for your health and safety lies within your own hands. By being an active participant in health and safety issues in your lab, your work and research experience will be as safe as possible.

The Principal Investigator and/or lab manager are responsible for training personnel on the potential hazards of the agents/infectious materials that they work with and the techniques to be used to handle the materials safely. The Biosafety Officer may be contacted at 348-3387/2621 for consultation and assistance in designing training programs as well as for facility design issues.

## 2.5 HANDWASHING

Prevention of transmission from person-to-person and contamination of the environment is accomplished by good laboratory practices. The most important of these is good hand-washing techniques. The following are recommendations for hand-washing in the laboratory environment.

Hands should be washed after removing gloves and after leaving the room in which work was conducted.

Hand washing technique:

- **Step 1:** Use warm running water,
- **Step 2:** Use enough soap
- **Step 3:** Use enough friction

- **Step 4:** Do not rush the process
- **Step 5:** Rinse well
- **Step 6:** Dry hands thoroughly with disposable paper towel
- **Step 7:** Turn off faucet with paper towel.

### 2.6 PERSONAL PROTECTIVE EQUIPMENT

Working in a laboratory setting poses several risk exposures to different materials in the environment, being equipment or biohazardous materials that may be used on a daily basis. Protection from several environmental exposures is of paramount importance when working in the laboratory.

All Supervisors are required to provide and assure that personal protective equipment is being used by their employees when there is a risk of occupational exposure. Personal protective equipment includes, but is not limited to, gloves, gowns, lab coats, head and foot coverings, and face shields and/or masks. All PPE should be selected based on the hazard. See the FIU Lab Safety Manual for the recommended types of PPE. The Supervisor's responsibilities include the following:

- Ensuring that the correct PPE is available and that all employees are trained in its usage.
- Providing for disposal of all soiled disposable PPE. Non-disposable lab coats me be washed in a washing machine.
- Replacement and/or repair of any malfunctioning PPE
- Gloves will be required to be worn when there is a potential for hands to come into contact with potentially infectious materials such as blood, biohazardous materials, etc. Gloves protect the individual when there may be cause for exposure through mucous membranes, non intact skin, etc.
- Gloves will be changed as soon as soiled and before leaving the laboratory area.
- Disposable gloves shall not be re-used.
- Whenever splashes or splatter are a possibility, face shields should be worn in order to prevent exposure to eyes, nose or mouth.
- Appropriate protective clothing shall be used by the individual when risk of splashes/splatters exists.
- **No sandals or open-toed shoes are allowed inside the laboratory area.**

### 2.7 SAFETY EQUIPMENT (PRIMARY BARRIERS)

The Biological Safety Cabinet (BSC) is the most commonly used device to help prevent exposure from splashes or aerosols created during handling of infectious agents. Other types of devices include enclosed containers as well as engineering controls designed to minimize exposures.

Personal protective equipment (PPE) is used in combination with BSCs and other engineering controls. PPE includes, but is not limited to: gloves, coats, shoe covers, gowns, boots, respirators, face shields, safety glasses, and/or goggles.

In all circumstances, PPE must be provided as just one of many primary barriers for prevention of exposure to infectious materials while working inside the laboratory.

### 2.8 FACILITY DESIGN AND CONSTRUCTION (SECONDARY BARRIERS)

Any recommendations for secondary barriers will depend on the risks involved with the actual agents used. Facility design helps to prevent the spread of a potentially biohazardous agent to the outside of the laboratory environment. When deciding on construction issues, laboratory management must take into consideration which agents are to be used and the proper containment parameters for such agents. Any deviation from standard uses or addition of different agents must also be considered.

### 2.9 SIGNAGE

Each laboratory is responsible for posting appropriate hazards signs outside of the laboratory doors to inform employees and visitors of the hazards within. All areas of the lab which use or contain biohazardous materials must, at the minimum, have the biohazards symbol posted outside the laboratory doors.

The following is the international biohazard symbol and is represented in orange or red:



### 3.0 RISK ASSESSMENT

The Principal Investigator and/or the Lab Manager must determine in which Risk Category the microorganism that they work with belong. There are several aspects one must look at in order to determine this:

- Agent's biological and physical nature.
- Pathogenicity
- Infectious dose
- Host susceptibility
- The procedures that may disseminate the agent.
- The best method to effectively inactivate the agent.

Risk groupings of infectious agents, 1 through 4, correspond to biosafety levels (BSL1-4, see below) which describe recommended containment practices, safety equipment and facility design features necessary to safely handle these pathogenic microorganisms. Pathogenic microorganisms include bacteria, viruses, fungi, parasites and other infectious agents.

Use of toxins and their potential for causing harm must also be taken into consideration when deciding on appropriate safety measures to take when working with these substances.

It is the responsibility of the Laboratory Manager and Principal Investigator(s) working in the lab to determine the risks involved in using the various biohazardous agents. In conjunction the Biosafety Officer, the Lab Manager and PI will write protocols for safe handling of potentially biohazardous agents as well as determine safe practices and necessary primary and secondary barriers which may be needed.

The following are excellent resources to use when determining Risk Categories for various agents:

The risk group listing of the *NIH Guidelines* at: (need new website link)

The American Biological Safety Association at:

<http://www.absa.org/riskgroups/>

### 3.1 LABORATORY SECURITY AND EMERGENCY RESPONSE

The microbiology laboratory in an academic institution presents several challenges in the area of laboratory security. There may be many individuals with access to the laboratory, and security background checks and other forms of pre-employment

verifications may not be feasible. The following paragraph is based on an excerpt from the CDC's *Biosafety in Microbiological and Biomedical Laboratories 5<sup>th</sup> Edition*. Basic laboratory measures can be used for experiments that involve BSL-1 and BSL-2 type microorganisms. These guidelines will help instructors and laboratory managers develop plans for security and emergency response purposes in the laboratory. The majority of the guidelines deal with Select Agents, but the basic principles should be applied to all types of laboratory environments to help ensure the safety of the people in the laboratory environment as well as the safety of the research activities.

To begin, the person in charge of the laboratory should consider the following points:

- Recognize that laboratory security is related to but different from laboratory safety.
- Control access to areas where biologic agents or toxins are used and stored.
- Know who is in the laboratory area
- Know what materials are being brought into the laboratory area.
- Know what materials are being removed from the laboratory area.
- Have an emergency plan.
- Have a protocol for reporting incidents.
- Follow your procedure

In recent years, concern has increased regarding use of biologic materials as agents of terrorism, but these same agents are often necessary tools in clinical and research microbiology laboratories. Traditional biosafety guidelines for laboratories have emphasized use of optimal work practices, appropriate containment equipment, well-designed facilities, and administrative controls to minimize risk of worker injury and to ensure safeguards against laboratory contamination.

Risk assessments for laboratories using biological agents should include:

- Systematic, site-specific reviews of physical security
- Security of data and electronic technology systems
- Employee security
- Access controls to laboratory and animal areas
- Procedures for agent inventory and accountability
- Shipping/transfer and receiving of select agents
- Unintentional incident and injury policies
- Emergency response plans policies that address breaches in security

The security plan should be an integral part of daily operations. All employees should be well-trained and equipped, and the plan should be reviewed at least annually.

### 4.0 BIOSAFETY LEVELS

#### 4.1 OVERVIEW

Four biosafety levels (BSLs) are described below. They are put forth as recommendations by the Centers for Disease Control (CDC) and the National Institutes of Health (NIH). They consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the operations performed the documented or suspected routes of transmission of the infectious agents, and the laboratory function or activity.

The Principal Investigator (P.I.) is specifically and primarily responsible for assessing the risks and appropriately applying the recommended biosafety levels. Generally, work with known agents shall be conducted at the biosafety level recommended. 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.

**IMPORTANT!!!** FIU currently does not have the facilities to support BSL3 and BSL4 level research. Therefore, BSL 3 and BSL 4 research is prohibited on FIU campuses.

#### 4.2 BIOSAFETY LEVEL 1

Biosafety Level 1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for hand-washing.

- Involves practices, safety equipment, and facility design and construction are appropriate for undergraduate and secondary educational training and teaching laboratories, and for other laboratories in which work is done with defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans.

#### 4.3 BIOSAFETY LEVEL 2

Even though organisms routinely manipulated at Biosafety Level 2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment, or in devices such as a BSC or safety centrifuge cups. Other primary barriers should be used as appropriate, such as splash shields, face protection, gowns, and gloves.

Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials. Extreme caution should be taken with contaminated needles or sharp instruments. Secondary barriers such as hand-washing sinks and waste decontamination facilities

must be available to reduce potential environmental contamination. Also, eyewash stations are highly recommended for mucous exposure incidents.

- Involves practices, equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, and other laboratories in which work is done with the broad spectrum of indigenous moderate-risk agents that are present in the community and associated with human disease of varying severity.
- With good microbiological techniques, these agents can be used safely in activities conducted on the open bench, provided the potential for producing splashes or aerosols is low.
- Hepatitis B virus, HIV, *Salmonellae* spp., and *Toxoplasma* spp. are representative of microorganisms assigned to this containment level.

### 4.4 BIOSAFETY LEVEL 3

At Biosafety Level 3, more emphasis is placed on primary and secondary barriers to protect personnel in contiguous areas, the community, and the environment from exposure to potentially infectious aerosols.

- Involves practices, safety equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection.
- *Mycobacterium tuberculosis*, St. Louis encephalitis virus, and *Coxiella burnetii* are representative of the microorganisms assigned to this level. Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols.

### 4.5 BIOSAFETY LEVEL 4

The Biosafety Level 4 facility itself is generally a separate building or completely isolated zone with complex, specialized ventilation requirements and waste management systems to prevent release of viable agents to the environment.

The primary hazards to personnel working with Biosafety Level 4 agents are respiratory exposure to infectious aerosols, mucous membrane or broken skin exposure to infectious droplets, and autoinoculation. All manipulations of potentially infectious diagnostic materials, isolates, and naturally or experimentally infected animals, pose a high risk of exposure and infection to laboratory personnel, the community, and the environment.



- Involves practices, safety equipment, and facility design and construction are applicable for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy.
- Agents with a close or identical antigenic relationship to Biosafety Level 4 agents also must be handled at this level. When sufficient data are obtained, work with these agents may continue at this level or at a lower level.
- Viruses such as Marburg or Congo-Crimean hemorrhagic fever are manipulated at Biosafety Level 4.

### 4.6 ANIMAL BIOSAFETY

Four biosafety levels are also described for activities involving infectious disease work with experimental animals. These four combinations of practices, safety equipment, and facilities are designated Animal Biosafety Levels 1, 2, 3, and 4, and provide increasing levels of protection to personnel and the environment. Refer to the CDC's *Biosafety in Microbiological and Biomedical Laboratories 5<sup>th</sup> Edition* for further information and agent categorization.

### 4.7 PLANT BIOSAFETY

Biosafety containment principles apply also to those individuals who work with plants and plant pathogens. Principle investigators should take be conscientious of the following biosafety practices concerning plant handling:

- Steps should be taken to avoid unintentional transfer of plant genes to other plants
- Prevention of the introduction of exotic plants into a new habitat which may affect the environment
- Prevention of harmful effects to the environment outside of the laboratory due to experiments being conducted using plant materials

Plant research involving plant diseases, plant-associated microorganisms, and other toxic or harmful entities is regulated by the Florida Department of Agriculture and Consumer Services. Other regulatory agencies may include the Food and Drug Administration, the Environmental Protection Agency, and the Centers for Disease Control and Prevention. Refer to the following websites for any rules or regulations that may involve this type of research:

[www.doacs.state.fl.us](http://www.doacs.state.fl.us)

[www.aphis.usda.gov](http://www.aphis.usda.gov)

[www.cdc.gov](http://www.cdc.gov)

[www.epa.gov](http://www.epa.gov)

[www.fda.gov](http://www.fda.gov)

Plant research involving the use of recombinant DNA is regulated by the National Institute of Health. Guidelines are provided at [www4.od.nih.gov/oba/rac/guidelines/guidelines](http://www4.od.nih.gov/oba/rac/guidelines/guidelines)

Plant research involving pathogens and/or recombinant DNA **must be approved** by the FIU Institutional Biosafety Committee and researchers should take into consideration all regulatory agency requirements for use and experimentation.

### 4.8 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 Manager to establish standard procedures that realistically address the issue of the infective hazard of clinical specimens.

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 (Class I or II) shall be used when performing procedures that might cause splashing, spraying, or splattering of droplets.

### 5.0 LABORATORY PROCEDURE GUIDELINES

#### 5.1 TRAINING

Training assures that those individuals working in a laboratory environment are working in a safe and conscientious manner. The Department of Environmental Health and Safety offers training courses online through the Moodle Platform. These training courses meet the minimum requirements as stipulated by governmental regulations and are not meant to be all-encompassing in their content and subject topics.

It is a requirement that all training records for each individual be kept on-site in the laboratories. During laboratory inspections, these records should be available for review.

#### 5.2 STANDARD OPERATING PROCEDURES

Standard operating procedures (SOPs) should be developed within each laboratory where potentially biohazardous agents will be used. Laboratory staff should be trained in regard to each SOP on an annual basis, or whenever new procedures are added to the work regimen. Required elements of an SOP should include:

- Descriptive title defining purpose of operation
- Preparation and revision dates
- Identification of department/laboratory for which the SOP is applicable
- Brief statement of purpose
- Identification of potential undesirable outcomes
- Identification of regulatory standards that apply to procedures
- Listing, by category of materials, tools, and equipment required to complete the SOP
- Definition of terms
- Prominent display of warnings and cautions prior to description of each task with potential danger involved
- Listing of all tasks included within SOP in sequential order

#### 5.3 MEDICAL SURVEILLANCE/MONITORING

Employees working with certain biohazardous agents/chemicals require periodic monitoring and evaluation, and employees may be eligible to participate in the FIU Medical Surveillance Program. For more information, refer to the EH&S website at: <http://ehs.fiu.edu>

### 5.4 RECORDKEEPING

#### **Biological Material Safety Data Sheets (MSDS)**

All laboratories performing research with known or potential biohazardous agents must maintain a comprehensive collection of a MSDS for each biohazardous agent. Questions or concerns regarding the MSDS of biohazardous materials should be directed to the Biosafety Officer at 348-3387.

#### **Exposure Control Plan (ECP)**

In accordance with the requirements of the OSHA Bloodborne Pathogen Standard (29 CFR 1910.1030), laboratories involved in research where there is a potential exposure to bloodborne pathogens are required to develop and maintain an ECP. The Department of Environmental Health and Safety has developed a model university ECP which provides a uniform policy for protection of university personnel who, as part of their job responsibility, anticipate exposure to bloodborne pathogens. The **Model Exposure Control Plan** is included in section 8.0 of the FIU Bloodborne Pathogen Exposure Control Plan.

#### **Engineering Control Devices and Safety Equipment, Testing/Certification**

Maintenance of testing and certification of all engineering control devices and safety equipment is necessary for achieving compliance with regulatory requirements. An abbreviated list of equipment requiring regular testing would include:

- Biological Safety Cabinets: annual testing/certification scheduled by EH&S
- Chemical fume hoods: annual testing provided by EH&S
- Autoclave units utilized for on-site treatment of biohazardous waste: monthly testing and regular documentation required

#### **Medical Surveillance Program**

Principal investigators must retain on file records of all vaccination/vaccination declination and medical approval required for all employees involved in tasks with risks of exposure to biological agents. Medical Surveillance records must be maintained on file for the duration of employment plus 30 years.

## **6.0 LABORATORY EQUIPMENT**

### **6.1 SELECTION**

When selecting new equipment for the laboratory, it is important to take the following recommendations into consideration:

- The instrument should limit contact between the user and the infectious agent.
- The equipment should be corrosion-resistant and should be easily cleaned/disinfected.
- Has no sharp edges or sides which may cause the operator harm.
- Manufacturer's recommendations for maintenance, cleaning, etc. should always be followed.
- Training on the proper use of the equipment for all employees who will operate it.
- All equipment should be decontaminated after each use, at minimum at the end of the day, and before it is sent out for repairs, etc.

### **6.2 AEROSOL GENERATION**

The main goal of biosafety is “containment” of any infectious materials that may pose a threat to persons inside and outside of the laboratory. Control of creation of aerosols is of paramount importance in helping to minimize exposure to harmful pathogens. Aerosols are more commonly created when manipulation of liquid suspensions, such as bacterial stock cultures, is being performed.

Shakers, sonicators and other mixing apparatus can cause significant amounts of aerosols to be created during routine operations. For this reasons, use of these instruments should be limited to Biological Safety Cabinets, especially when infectious organisms are being used. When using these instruments it is important to remember the following safety considerations:

- Check the condition of the instruments before use.
- Allow aerosols to settle for at least a minute before opening or after mixing will help minimize exposure.
- Disinfect all surfaces after use

### **6.3 CENTRIFUGES**

Centrifuges are one of the most commonly used equipment in the research laboratory. However, the use of centrifuges presents a risk for laboratory workers due to the creation of aerosols. Damage to or improper maintenance of the centrifuge may cause breakage of materials during the operation of the instrument, leading to the generation of large amounts of aerosols which may pose a threat to the laboratory worker. Performing the following steps can help minimize this scenario:

- Inspect the centrifuge on a regular basis to look for signs of cracks or hairline fractures which could indicate stress on the instrument.
- Perform quality control on the centrifuges on a regular basis to make sure that it is performing up to manufacturer's standards.
- Avoid filling the tubes placed inside the centrifuge to the rim.
- If centrifuge buckets are available, place tubes and close and open them inside a Biological Safety Cabinet before loading them into the centrifuge.
- When capping the tubes, use caps or stoppers, trying to avoid lightweight material such as aluminum foil, which can fall off during operation of the instrument.
- Always be sure that the centrifuge is properly balanced before each use.
- Do not open the lid immediately after operation of the instrument. Wait about a minute or so to allow any possible aerosols what were formed to settle down
- If a break does occur inside the centrifuge, stop the centrifuge immediately and wait at least 10 minutes before opening the lid to clean up the materials inside. Wear proper PPE when cleaning up the materials.

### 6.4 PIPETTES

When used improperly, pipettes have the potential for product contamination as well operator injury. There are various types of pipettes to use in the laboratory setting ranging from plastic to glass.

When working with infectious materials that produce aerosols when manipulated, all pipetting should be performed inside a Biological Safety Cabinet to ensure that exposure to the operator is minimized.

Minimization of splashing and formation of aerosols when using pipettes can be accomplished by using the following techniques:

- Always check pipettes and pipettors before using them. Look for cracks, chips or any other defects that may interfere with the normal operation of glass/plastic pipette.
- Make sure to keep the pipette upright throughout the procedures performed, especially when the liquid is inside the pipette.
- When expelling the liquid from inside the pipette, do it gently right above the target area or let the liquid run down the sides of the container if applicable.
- Do not force out the liquid too quickly.
- When finished using re-usable pipettes, place in the disinfectant solution in a horizontal fashion to avoid expelling of the liquid from either end which may cause formation of aerosols.

### 6.5 OTHER EQUIPMENT

The following is a list of other types of equipment found in the laboratory with handling and maintenance suggestions. The list should not be taken as an all-inclusive listing of laboratory equipment.

- Microscopes: Clean and wipe down with appropriate wipes and disinfectant after each use. Disinfectant should be applicable to the pathogen being used as well as non-corrosive to the instrument.
- Water baths: Clean/disinfect on a regular basis (at least once a week). To prevent electrical shock, make sure to unplug the unit before removing or adding water to the system.
- Tissue grinders: Always use inside a biological safety cabinet
- Transfer loops: Disposable one-time-use is best to help minimize use of flames and gas inside the laboratory. If re-usable ones are used, make sure to take steps to help minimize potential formation of aerosols when streaking or flaming the loops.

### 7.0 BIOLOGICAL SAFETY CABINETS (BSC)

#### 7.1 INTRODUCTION

Biological Safety Cabinets (BSC) are primary barriers used to prevent exposure or contamination of biohazardous or infectious agents. A BSC surrounds the immediate area where work is being done, but it does not assure that some aerosols may not escape.

#### 7.2 BIOLOGICAL SAFETY CABINET CLASSIFICATIONS

There are four classes of BSCs: Class I, Class II-Type A, Class II-Type B, and Class III. All four classes are suitable for work with biohazardous materials in BSL 1 to BSL 3. The Class III BSC is required for BSL 4 work. The following provides a brief overview of the types of BSCs available for use in laboratories. A more thorough explanation for usage and maintenance can be found at the NIH website: <http://www.niehs.nih.gov/odhsb/biosafe/bsc/bsc.htm>.

##### **Class I**

The Class I BSC functions to protect the user and the environment, but not the experiment. Therefore it accommodates experiments using equipment such as sonicators, shielded centrifuges, blenders, and mixers. The cabinet is a partial containment unit. Sudden withdrawal of hands may compromise the airflow and may expose the user to aerosols. Since the user's hands and arms are not protected from contact with the hazardous materials, the use of Personal Protective Equipment is of utmost importance.

##### **Class II**

The Class II BSC is a front opening cabinet with inward air flow to protect personnel, the work, and for environmental protection. These are more commonly referred to as laminar air flow hoods. There are two types of Class II BSCs, A and B, that differ principally as to vertical dimensions of the front opening, proportion of air recirculated, velocity of inflow air, manner of exhaust discharge, and whether contaminated air plenums are under positive pressure.

Due to the high percentage of air recirculation, Type A BSCs are restricted to work with BSL level 1 to 3 agents in the absence of volatile or toxic chemicals and radionuclides. The type B cabinets allow work with toxic chemicals and radionuclides. The type B BSCs are further sub-typed to types B1, B2, and B3. The Class II BSCs are partial containment units subject to the same barrier-function compromises as the Class I.



### **Class III**

The Class III BSC has enclosed ventilated cabinets which offer the highest degree of personnel and environmental protection. In addition, the experiment is protected because manipulations of cultures are performed through attached rubber gloves.

The Class III BSC can be compromised by punctures of the gloves or the use of highly volatile materials that contribute to the decline of the negative pressure barrier. Flammable gas should not be piped to these units.

BSCs must be clearly labeled with the class and type, the date of the last performance test, the name of the person performing the test, the company name, and a certification of performance. All personnel working with BSCs should be appropriately trained in their used to assure proper handling of these cabinets and prevention of exposure to materials that are used.

### **7.3 BSC PROCEDURAL GUIDELINES**

- The BSC should be on at all times while working inside. Once the work has finished, the motor may be turned off.
- If the BSC has been left off, turn it on and let it purge the air for at least 3 minutes before beginning to work inside it.
- The UV light should be off when someone is working inside the cabinet as it can cause eye and skin damage.
- The UV light does provide for some sterilization of the environment when work is finished, but it should not be used alone to assume that sterilization is complete. Proper sterilization/disinfection techniques should be applied.
- Place all necessary items inside the cabinet before beginning work. There should not be any clutter inside that may impede the flow of air inside the cabinet and by having all materials inside, the employee is less likely to have to move in and out to get materials needed. This prevents interruption of the air flow as well.
- A small waste container should be placed inside for any disposable materials.
- No materials should be placed on the air-intake valves to avoid impeding the air flow.
- While working inside the BSC, always wear lab coats and gloves
- Try to limit arm motions and quick, rapid movements inside the cabinet as these may cause a disturbance in the air flow pattern and could let aerosols escape and cause possible exposure.
- Do not use a Bunsen burner inside the BSC as this will impede the air flow inside the BSC. If a procedure calls for the use of a flame, use a burner with a pilot light and place it to the rear of the work space where any type of air turbulence will have a minimal effect.
- Large objects such as a centrifuge that are placed inside the BSC may impede the air flow.

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## 7.0 BIOLOGICAL SAFETY CABINETS

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- If an accident occurs during a procedure, immediately clean the area with appropriate disinfectant solution. Leave the BSC on during this time to minimize exposure to aerosols.
- It is important to remember that a BSC is not the same as a chemical fume hood and should not be used for both purposes.
- Once the work is completed, close up all waste containers and organize materials used.
- Allow the cabinet to operate for five more minutes to finish purging the air inside.
- Remove all materials from inside the cabinet and decontaminate the work surface.
- The UV light may be turned on.
- When not in use the BSC should not be used for storage of materials.
- When cleaning/decontaminating the BSC, wipe down the decontaminated area with water after using the disinfectant (i.e. 10% bleach, etc.), to prevent corrosion of the BSC.

### 7.4 LAMINAR FLOW HOODS

Laminar flow clean benches are not BSCs. They discharge HEPA-filtered air across the work surface and toward the user. This type of engineering control only protects the product. They can be used in dust-free assembly of activities for items such as electronics and sterile equipment. **These benches should never be used when handling cell cultures or infectious agents.**

### 7.5 INSPECTION/CERTIFICATION REQUIREMENTS

As part of a good quality control program and to assure that the BSC is working properly, all BSCs must be inspected and certified under the following circumstances:

- When it is newly installed
- After the filter has been replaced or the motor has been changed
- After being moved (even if moved within the same room)
- At least annually

The Department of Environmental Health and Safety is responsible for the scheduling of annual inspections for the Biological Safety Cabinets. However, Supervisors must notify EH&S when they have purchased, relocated, or repaired a biosafety cabinet so that arrangements can be made. **Replacement or repairs of the equipment are the financial responsibility of the person/department responsible for the BSC. Any additional inspections that are needed will also be the responsibility of the department/person requesting the inspection.**

### 8.0 BIOHAZARDOUS/BIOMEDICAL WASTE

The following guidelines will ensure a safe working environment for all employees/students of Florida International University. The information provided deals with proper handling and disposal of biomedical waste products produced from activities on university grounds.

#### 8.1 RESPONSIBILITIES

- The principal investigator has a responsibility to his/her colleagues, students and to the community to ensure that laboratory waste handling procedures and policies are well defined and appropriately followed. These wastes may pose a risk to those who generate it, those who transport it, and those who treat it.
- The laboratory is responsible for the containment of hazardous wastes requiring special handling; and, it is also responsible for the development of appropriate policies and procedures, which must become an integral part of the laboratory's standard operating procedures manual.
- Proper disposal and follow-up procedures will help ensure that hazards are minimized or eliminated altogether.

#### 8.2 CLASSIFICATION

- Cultures and stocks of infectious agents and associated biologicals. These include cultures from medical and pathological laboratories; cultures and stocks of infectious agents from research and industrial laboratories; wastes from the production of biologicals, discarded live and attenuated vaccines; and culture dishes and devices used to transfer, inoculate, and mix cultures.
- Pathological wastes including tissues, organs, and body parts that are removed during surgery or autopsy.
- Waste from human blood and products of blood including serum, plasma, and other blood components.
- Sharps that have been used in patient care or in medical research, or industrial laboratories, including hypodermic needles, syringes, Pasteur pipettes, broken glass, and scalpel blades.
- Contaminated animal carcasses, body parts, and bedding of animals that were exposed to infectious agents during research, production of biologicals, or testing of pharmaceuticals
- Wastes from surgery or autopsy that were in contact with infectious agents. The following include: soiled dressings, sponges, drapes, lavage tubes, drainage sets, underpads, and surgical gloves.
- Laboratory wastes from medical, pathological, pharmaceutical, or other research, commercial, or industrial laboratories that were in contact with infectious agents, including slides and cover slips, disposable gloves, laboratory coats, and aprons

- Dialysis wastes that were in contact with the blood of patients undergoing hemodialysis, including contaminated disposable equipment and supplies such as tubing, filters, disposable sheets, towels, gloves, aprons, and laboratory coats
- Medical equipment and discarded parts that were in contact with infectious agents.
- Biological waste and discarded materials contaminated with blood, excretion, exudates or secretion from human beings or animals isolated to protect others from communicable diseases.
- Such other waste material that results from the administration of medical care to a patient by a health care provider and is found by the Administrator to pose a threat to human health or the environment
- All types of wastes that have been autoclaved.

### 8.3 DISPOSAL

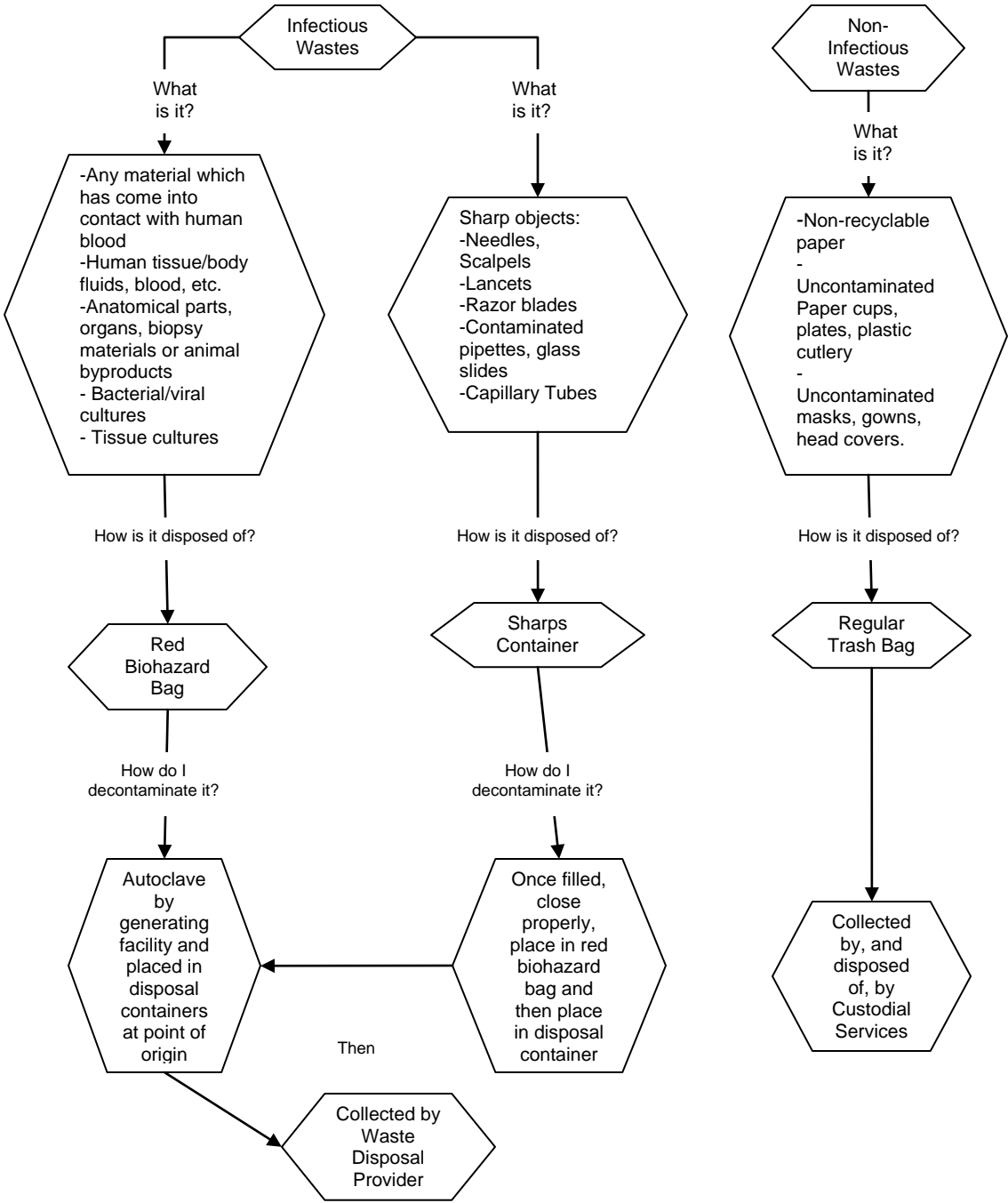
- At the point of origin, the non-sharps medical waste shall be placed into impermeable red plastic bags, the bags sealed and placed into cardboard boxes supplied by the medical waste transporter.
- Contaminated sharps waste will be immediately discarded in containers that are closable, puncture-resistant, leak-proof and labeled with the Biohazard symbol. (Departments are responsible for purchasing of their own sharps containers).
- Throughout the time of use maintain the container within easy reach, keep upright and make sure that the container is not overfilled
- Close the container before removal and replacement
- Sharps containers shall be sealed and placed into red bags then placed in a secondary container
- The secondary container (larger container where the sharps containers and other wastes go) will be closeable, constructed to contain all the contents that are placed inside and leak-proof, labeled as biohazardous.
- Red bags and outer cardboard boxes shall be labeled with the generator's name and address.
- Red bags and outer cardboard boxes shall be identified with the international biological hazards symbol printed on the box.
- All containers will be closed prior to removal from the area/facility.

*Note: Some facilities on campus (research laboratories, etc.) have the capability of decontamination/sterilization of their biohazard/biomedical wastes by autoclaving the materials. When this is the case, they shall decontaminate/autoclave the materials and then discard properly as above to set up for proper pick-up and disposal*

### 8.4 STORAGE AND LABELING

- All containers/bags must be labeled with the generator's name and time and date
- FIU generating facilities shall not store biomedical/biohazardous wastes for more than 30 days.
- Biomedical waste shall remain at the generating facility until the transporter picks up the waste.
- Indoor storage areas must have restricted access, shall be located away from pedestrian traffic, be vermin and insect free, and shall be maintained in a sanitary condition.
- Outside storage areas (containers) shall be conspicuously marked with the international biohazards symbol and shall be secured against vandalism and unauthorized entry. (the symbol should be at least 6 inches in diameter)
- Transfer of biohazardous/biomedical waste within the generating facility shall be conducted safely and with appropriate care.
- Minimum protective equipment for handling biomedical waste is disposable gloves.
- Biohazardous/Biomedical waste shall NOT be transferred from the generating facility to other buildings on campus.

**Biohazardous Waste Disposal**



### 9.0 BIOHAZARDOUS SPILLS

Any spill involving high-risk Category 2 microorganisms should be reported immediately to the Biosafety Officer at 305-348-3387.

### 9.1 BIOHAZARDOUS SPILL KIT

Microbiological and research laboratories should prepare and have readily available a biohazardous spill kit inside each lab. Spill kits are needed for laboratories working with microbiological agents at the BSL-2 level and above, as well as for those who work with large amounts (> 1 Liter) of BSL-1 material. The minimum contents of the spill kit should include the following:

- EPA-approved disinfectant
- Spray bottle
- Forceps or other mechanical means of picking up sharps items
- Paper towels
- Red Biohazard bags for collection of contaminated items
- Proper PPE including gloves, laboratory coats, eye wear, face shield (if needed)

Regular household bleach is a standard disinfectant. Other disinfectants could be used to clean up any spills in a biological laboratory. The type of disinfectant used should be effective on the types of agents used. Disinfectants must be registered with the Environmental Protection Agency and should take into regard regulations put forth by OSHA Bloodborne Pathogens Standard as well.

When using bleach as a disinfectant, prepare a 10% solution by mixing 1 part bleach to 9 parts water. Always prepare fresh when cleaning up a biohazard spill. When using a different type of disinfectant, follow the manufacturer's recommendations/guidelines for use.

### 9.2 EXPOSURE

The following are recommendations for what to do in case of an exposure:

- **Intact Skin**
  1. Remove contaminated clothing
  2. Wash contaminated skin for at least 1 minute with soap and water
- **Needle-stick or other puncture wounds** (i.e. cut with a scalpel, etc.)
  1. Wash the affected area with soap and water for at least 5 minutes
  2. Seek medical attention as soon as possible
  3. Inform Supervisor of incident

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## 9.0 BIOHAZARDOUS SPILL AND EXPOSURE RESPONSE

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- **Mucous membrane splashes** (i.e. splash to eyes, nose, mouth, etc.)
  1. Immediately flush out eyes with water at least 15 minutes using an eyewash station.
  2. Hold open eyelids during this time and rotate eyes back and forth
  3. Seek medical attention as soon as possible
- **Ingestion or inhalation**
  1. Seek medical attention as soon as possible
  2. Do not induce vomiting unless directed to do so by a health care provider

### 9.3 SMALL BIOHAZARDOUS SPILL (OUTSIDE BSC)

- Appropriate Personal Protective Equipment, including disposable gloves, lab coat, etc. must be worn throughout the clean up procedure.
- If the spill involves broken glass or sharps, DO NOT pick these up by hands. Use mechanical means such as forceps or pan brush (which will be decontaminated later) to pick up pieces and then dispose of appropriately.
- Cover the spill with paper towels and carefully pour an appropriate disinfectant around the spill. Try not to create any aerosols while performing this task.
- Cover the spill with disinfectant soaked paper towels and let stand for 20 minutes.
- Once the 20 minutes have expired, collect all materials and dispose of in an autoclave bag for decontamination/sterilization.
- Remove contaminated gloves, clothing and dispose of as well.
- Thoroughly wash hands and face with soap and water.

### 9.4 LARGE BIOHAZARDOUS SPILL (OUTSIDE BSC)

- Hold your breath while exiting, alert co-workers and leave the lab area immediately.
- Close the laboratory doors and post a warning sign to not enter the laboratory.
- Remove any contaminated clothing and wash hands as soon as possible.
- Leave the laboratory area for at least 30 minutes to allow for any aerosols that might have formed to settle down.
- Before returning to the laboratory, put on appropriate Personal Protective Equipment such as gloves and lab coat and respirator mask when working with high-risk Category 2 microorganisms. This should be done in a “clean” area designated for this.
- Place paper towels on the contaminated area and pour disinfectant solution *around* the area not directly on top of the spill.
- Place disinfected-soaked paper towels on the spill as well and let stand for approximately 20 minutes.
- Transfer all contaminated materials into an autoclave bag for decontamination.



## **9.0 BIOHAZARDOUS SPILL AND EXPOSURE RESPONSE**

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- Wash and mop the contaminated area to assure proper removal of materials/disinfectant.
- Remove and place PPE into autoclave bag as well. This should be done in a separate area so that cross-contamination does not occur.
- Thoroughly wash hands and face and other contaminated areas, if needed, with soap and water.

### **9.5 BIOHAZARDOUS SPILL (INSIDE BSC)**

- Leave the biosafety cabinet on during the clean-up process.
- Put on appropriate PPE.
- Place paper towels on the spill.
- Apply appropriate decontamination solution around the spill.
- Cover the spill with disinfectant-soaked paper towels.
- Spray walls and work surfaces inside the BSC with appropriate disinfectant.
- Let stand for 20 minutes.
- Drain excess solution into cabinet base.
- Lift out the tray and removable exhaust grille work.
- Clean the top and bottom surfaces as well.
- Take out paper towels and all contaminated materials and place in an autoclave bag for appropriate disposal/decontamination.
- Drain decontamination solution from cabinet base and dispose of appropriately.
- Dispose of contaminated PPE. This should be done in a special designated area to prevent cross-contamination of the room.
- Thoroughly wash hands and face and other contaminated areas, if needed, with soap and water.

### **9.6 RADIOACTIVE MATERIAL SPILL**

When cleaning up a biohazardous material that contains radioactive materials, use procedures that protect from both types of hazards, i.e. PPE for biohazardous materials as well as for radioactive materials.

The biohazard aspect of the spill should be addressed before cleanup is initiated. Also, the type of radionuclide should be taken into account and the Radiation Safety Officer should be informed before any cleanup procedures are initiated.

The biohazardous material must be completely inactivated before radiation cleanup protocols/procedures are initiated. To clean up this type of spill:

- Follow recommendations for basic biohazard spill cleanup (wearing radiation protection PPE while cleaning up)

## **9.0 BIOHAZARDOUS SPILL AND EXPOSURE RESPONSE**

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- DO NOT use bleach solutions as the disinfectant for this type of spill as it may create a radionuclide gas in the process. Use another type of disinfectant such as a phenolic or iodophor.
- Once the cleanup is completed, place materials in a biohazard bag and place it inside an approved radioactive waste container and label according to Radiation Safety guidelines.

**Note:** Approval from the Radiation Laser Safety Officer is required **PRIOR** to the steam sterilization/disinfection of the biohazardous/radioactive material. Refer to the FIU Radiation Safety Manual for more information on radioactive spills.

If, when working inside the Biological Safety Cabinet, there is a power outage and the lab is not equipped with backup generators, the lab workers should leave the area immediately and contact the Biosafety Officer. After power has been restored, decontamination of the work area may be deemed necessary.

### 10.0 LABORATORY INSPECTIONS

Laboratory inspections are a necessary part of maintaining a safe laboratory atmosphere and are required by the CDC and NIH for work in a biohazard laboratory.

### 10.1 RESPONSIBILITIES

The Biosafety Officer from the Department of Environmental Health and Safety will be responsible for conducting laboratory inspections. These inspections may be conducted on a quarterly, bi-annual, or annual basis depending on the hazard “ranking” of the lab. This hazard ranking involves issues such as type of biohazardous research involved as well as hazardous chemicals and other materials used.

The inspections will take place with the appropriate checklists (i.e. Biosafety Safety Inspection: Appendix A) used to determine compliance or non-compliance issues in each lab. PIs are encouraged to contact the Biosafety Officer before scheduled inspections to address any issues or concerns they may have.

Scheduling of the inspections will be initiated by the Biosafety Officer. Ample time will be given for preparation of the laboratory area prior to the inspection and any questions/issues should be addressed beforehand.

### 11.0 STERILIZATION/DISINFECTION

#### 11.1 INTRODUCTION

An important aspect of biohazard containment involves proper cleaning and disinfection of work areas and materials used in the laboratory. Improper cleaning procedures may lead to exposures of the laboratory workers as well as cross-contamination of the materials.

Emergency spill procedures and proper hand-washing techniques are among the controls that persons in the laboratory setting can use to ensure that proper containment is achieved.

When choosing what type of method for cleaning is to be used, the correct method to use depends on the following:

What are the targets organisms that you want to remove?

What are the characteristics of the area(s) to be cleaned?

#### 11.2 DISINFECTION

*Decontamination/Disinfection:* reducing the number of potentially pathogenic organisms by applying physical or chemical agents to the area that may be contaminated.

The following is a short review of possible disinfecting agents to use when cleaning in the laboratory. All types are not listed and not all disinfection agents apply to all types of organism including spores.

Type of Disinfectant	What it can be used for.
Alcohols	Use of ethyl or isopropyl alcohol at a concentration of 70-80% is good for general disinfection, but they are not effective against bacterial spores. Eye irritant, can be toxic if absorbed
Phenols	0.2-3% solution effective against vegetative bacteria, fungi, and viruses containing lipids; corrosive, skin irritant, eye irritant, resp. irritant, toxic if absorbed; not effective against spores (e.g Lysol, pine oil, cresol)
Formaldehyde	5-8% formalin is a good disinfectant against vegetative bacteria, spores, and viruses; skin irritant, eye irritant, resp. irritant, toxic if absorbed; known carcinogen
Quaternary Ammonium Compounds	Lipoviruses, not effective against bacterial spores; toxic if absorbed (e.g Parvasol, Maxima 128, A33)
Chlorine (liquid)	Low concentration (1:50 dilution) effective against

## 11.0 STERILIZATION AND DISINFECTION

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	vegetative bacteria and most viruses, but higher concentrations (1:10 dilution) are required for bacterial spores, and spills involving blood/organic materials; corrosive, skin irritant, eye irritant, resp., irritant, toxic if absorbed (e.g chlorine bleach)
Iodine	Vegetative bacteria and viruses, not as effective for bacterial spores; skin irritant, eye irritant. 75 ppm dilution recommended for disinfecting work surfaces.

Once the proper disinfection method has been chosen, it is important to follow these guidelines:

- Disinfect all work surfaces including floors cabinet tops and equipment on a regular basis
- Minimize the amount of materials that are around the area in which biohazardous materials are used. In other words, a clean and uncluttered work environment provides for safe use of these materials.
- Materials that are autoclavable or disposable should be used as often as possible.
- Sterilize or store all biohazardous materials when work is finished
- Chemical disinfectants may not work as well on some materials, so higher concentration or an extended contact time may be needed.
- When autoclaving wastes, it is important to attach an indicator (e.g. autoclave tape) to the outside of the bag to ensure proper sterilization of the contents.

### 11.3 STERILIZATION

*Sterilization:* Complete destruction of all living organisms in the contaminated area.

The most common and effective method for sterilization of biohazardous materials used in the laboratory is the Steam Autoclave. This instrument may be used to sterilize a variety of materials including glassware, media, wastes, instruments, etc.

In order to ensure proper containment practices and to prevent exposures of employees, proper use of the autoclave is mandatory as well as the control of waste management as prescribed by local, state, and federal regulations.

Several factors that must be taken into account when autoclaving to ensure proper sterilization including temperature needed, time and direct steam contact, removal of air from the chamber to ensure adequate time to achieve the sterilization.

### 11.4 GUIDELINES

- All potentially infectious materials used in the laboratory such as glassware, instruments, etc. must be autoclaved before being washed and stored or disposed of after use.
- Training of personnel who use the autoclave is mandatory to assure compliance with safety and use issues.
- Toxic, volatile, or radiological materials should not be autoclaved as this can pose a hazard to those using the autoclave as well as to the environment surrounding it.
- Biohazardous materials must not be left unsecured and should be autoclaved on a daily basis unless stored appropriately in the lab until the time that the material will be sterilized. Appropriate storage includes proper labeling of the waste materials as indicated by a biohazard symbol on the outside of the waste container/bag.
- All bags used for biohazardous materials must be labeled and made of material appropriate for containing biohazardous substances.
- Sharps such as broken glass or needles must not be placed inside the plastic biohazard bags.
- Label each bag with an indicator strip that will alert the handler if the sterilization process was successful or not.
- When loading the bags into the autoclave, place them in a shallow secondary container such as a metal or plastic pan to allow for proper steam accumulation
- Bags or other materials to be autoclaved should never be in direct contact with the bottom of the autoclave.
- Do not overload to allow for proper circulation of steam throughout the autoclave.
- Once properly closed the operation of the autoclave may begin
- The temperature and time for autoclaving varies by brand, etc. but generally 121 degrees Celsius at 15 psi for 30 minutes should be sufficient for adequate sterilization of the materials.
- A biological indicator should be routinely used, at least once a month, to ensure that the autoclave is operating according to standard.
- Investigators and users of the autoclave should develop their own SOP for the use of their particular autoclaves as models vary and temperature, psi requirements, and time may differ.
- After the sterilization process is complete, open the door of the autoclave, using appropriate protective wear such as heat-resistant gloves, approximately 0.5 inches to let the residual steam out.
- Wait approximately 10 minutes for this process to complete and to allow the contents to cool down somewhat.
- Care should be taken when removing the contents as spilled liquids, media, etc. could splash and cause burns to the person taking the materials out.

## **11.0 STERILIZATION AND DISINFECTION**

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- Place all autoclaved bags in the designated area for pickup by biohazardous waste services.
- All quality control measures and use of the autoclave must be documented and records must be kept and made available during inspections of the facilities.

### 12.0 SHIPPING BIOLOGICAL MATERIALS

#### 12.1 General

Procedures for receiving and unpacking infectious materials **shall** be established by the labs receiving these materials. Employees whose responsibilities include the receipt of packages should have specific instructions regarding infectious materials and training to recognize the hazardous nature of the material being received. Employees should also be able to recognize whether or not the material has been packaged, labeled and manifested or documented appropriately.

#### 12.2 Receipt of Infectious Materials

Shipments of hazardous materials must be received (generally) by those to whom it is addressed. This can be accomplished via a certified carrier such as Federal Express. University Mail Services is certified to accept and transfer hazmat packages from the U.S. Postal Service (USPS).

Employees receiving shipments are responsible for the following:

- Provide a designated and secure area of the laboratory for receipt of materials
- Use all appropriate personal protective equipment and containment devices (biological safety cabinet or chemical fume hood)
- Before accepting, inspect the parcel for leakage indicated by broken or improperly sealed containers. If the package is rejected (not accepted) due to leakage or other damage, the carrier will work with the shipper to resolve the problem. If the shipment is critical and must be accepted, EH&S should be contacted at 7-2621, and further activities should be conducted with care in an appropriate containment device.

#### 12.3 Transportation of Biohazardous Materials

All shipments of infectious or diagnostic specimens must be packaged, labeled, and transported in compliance with local, federal, and international transportation and shipping regulations, including U.S. Department of Transportation (DOT) (ground) and International Air Transport Association (IATA) (air) regulations. Personnel who package, handle, and ship these agents (including import and export) must receive applicable training.

#### 12.4 Packaging and Labeling

There are specific requirements for the packaging and labeling of biological materials. Biological materials include **infectious substances (etiologic agents)** and **biological specimens**. Proper shipping papers, "Shipper's Declaration for Dangerous Goods", must be completed for shipment of infectious substances, but not for biological specimens. Many diagnostic samples/specimens must be classified as infectious substances by virtue of the agent being tested. However, both groups of materials require proper packaging, labeling, and marking.



## 12.0 SHIPPING BIOLOGICAL MATERIALS

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Human blood always requires “Universal Precautions” and **may** be considered a diagnostic specimen or infectious substance depending upon the test or nature of the specimen.

If dry ice or liquid nitrogen accompanies a shipment, these materials must be declared, and packages must be properly labeled. Dry ice should **never** be placed in a sealed container or other container that **cannot** breathe.

Packaging requirements:

- Provide triple layers of containment for biological samples.
- Use proper internal labeling for identification of the samples and a full inventory.
- Provide internal absorbent materials in case of spills or leakage.
- Provide proper labeling on the outside of the package as to contents (class 6-2 diamond label, biohazard symbol, “infectious agents”, name and phone number of an authority if a dangerous good, etc.).
- Use a certified, UN-numbered container when appropriate. These containers have been tested and shown to withstand various drop and pressure tests.
- Use labels for all of the dangerous goods present, i.e. one for the biological agent as well as one for the dry ice with which it is shipped.

### Definitions

*Infectious substances or etiologic agent:* DOT definition: A viable microorganism or its toxin, that causes or may cause disease in humans or animals. IATA definition: Substances known to contain, or reasonably expected to contain, pathogens. Pathogens are microorganisms (including bacteria, viruses, rickettsia, parasites, fungi) or recombinant microorganisms (hybrid or mutant) that are known or reasonably expected to cause disease in humans or animals.

### *Biological specimen:*

Any human or animal material including excreta, secretions, blood, blood components, tissue, and tissue fluids being shipped for the purposes of diagnosis.

**NOTE:** that diagnostic specimens that are “known or reasonably expected” to contain pathogens must be handled as infectious substances.

### Procedure for packaging infectious substances

#### Primary container(s):

1. Use watertight/leak-proof vial, tube or plate made of glass, metal or plastic.
2. Identify the contents.
3. Reinforce screw caps and plates with adhesive tape, use a metal crimp seal or skirted stopper for metal and glass.

#### Secondary container:

1. Use a watertight container and reinforce with adhesive tape.
2. Affix biohazard label.

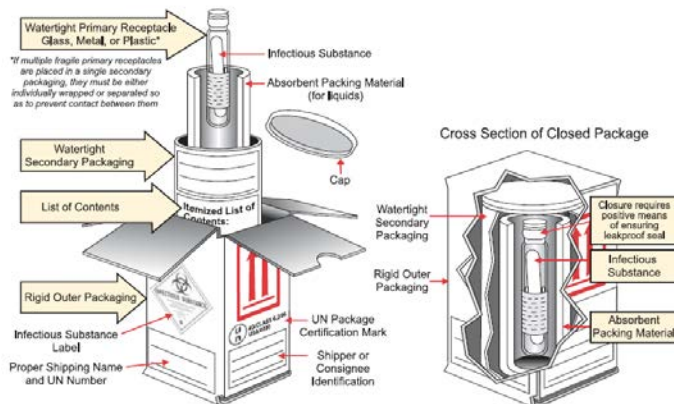
## 12.0 SHIPPING BIOLOGICAL MATERIALS

3. Affix a label with a complete list of the contents including the scientific name and the amount in milliliters.
4. Use absorbent packing material sufficient to completely absorb full contents of each primary container.

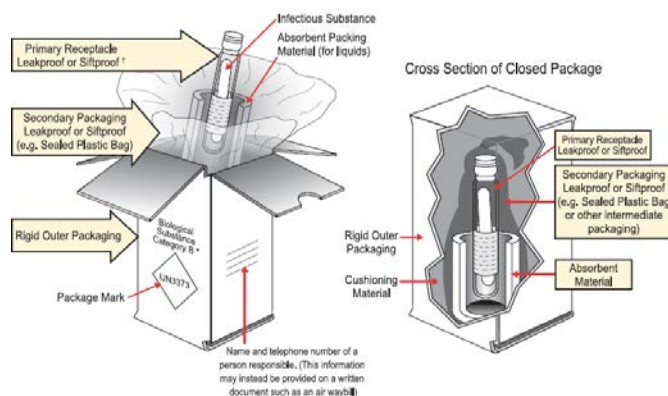
### Shipping container:

1. Use one made of fiberboard and certified for use with the agents/samples being shipped.
2. Affix proper labels.
3. Affix address label with the complete address and phone number for both the shipper and the recipient.
4. Affix the Class 6, Division 6.2 infectious substance label as required.
5. Affix a label with the UN number, proper shipping name, and amount of all hazardous substances.
6. Affix the double up arrows sticker.
7. Use the Class 9 hazardous material label if dry ice is used.
8. Complete the Shipper's Declaration for Dangerous Goods as required.

### Category A, Infectious Substance Packaging



### Category B, Biological Specimen Packaging



## **APPENDICES**

- A** BIOSAFETY CHECKLIST FORM
- B.** DIRECTORY OF SERVICE AND EMERGENCY PROVIDERS
- C.** TABLE OF BIOSAFETY LEVELS 1-4

**APPENDIX A**

**BIOSAFETY CHECKLIST FORM**

## BIOSAFETY LABORATORY CHECKLIST

LOCATION OF FACILITY INSPECTED: \_\_\_\_\_

DATE OF INSPECTION: \_\_\_\_\_

*Complete all items ("X" one column). A "Not Applicable" (N/A) column is provided because all items and conditions may not apply to each area.*

<b>AUTOCLAVES AND STERILIZERS:</b>	<b>YES</b>	<b>NO</b>	<b>N/A</b>
1. Are all safety valves and safety interlocks checked regularly?			
2. Is the floor gasket in good condition and sealing properly?			
3. Are temperature and pressure gauges legible and in good condition?			
4. Are drains clean and free flowing?			
5. Are protective gloves available for handling hot items?			
6. Are procedures for autoclaving liquids clearly posted?			
7. Are only authorized personnel allowed to use autoclaves and sterilizers?			
<b>BIOLOGICAL SAFETY PROGRAM:</b>			
1. Brucella, Chlamydia, Leptospira Laboratories, Biosafety Level 2			
2. Is access limited or restricted?			
3. Are work surfaces decontaminated daily?			
4. Are work surfaces decontaminated after any spill of viable material?			
5. Are bench tops impervious to water?			
6. Are all areas readily accessible for cleaning?			
7. Is a sink available for hand washing?			
8. Is an autoclave available?			
9. Are infectious wastes decontaminated?			
10. Is eating, drinking, and applying cosmetics prohibited in the work area?			
11. Are wastes stored in leak proof durable containers?			
12. Are warning signs posted?			
13. Is there an insect and rodent control program?			
14. Are laboratory coats, gowns, smocks, uniforms, or safety glasses worn in the laboratory?			
15. Are only needle-locking syringes or disposable syringes used?			
16. Are waste syringes and other sharps properly disposed of in puncture-resistant, leak-proof containers?			
17. p. Is a safety manual available?			
18. q. Are biological safety cabinets or other physical containment devices used for procedures with a potential for creating infectious aerosols?			
19. Is Biosafety Level 3 containment equipment available, where necessary?			
<b>PERSONAL PROTECTIVE EQUIPMENT:</b>			
1. Is the appropriate personal protective equipment available?			
2. Are employees trained in the handling, use, and care of the equipment?			
3. Are respirators stored in a convenient and sanitary location?			

4. Are respirators inspected routinely?			
5. Have employees who are required to wear respirators received medical approval?			
<b>INFECTIOUS WASTE:</b>	<b>YES</b>	<b>NO</b>	<b>N/A</b>
1. Is infectious waste contained and stored in a manner which affords protection from animals, weather, and does not provide a breeding place or food source for insects and rodents?			
2. Is infectious waste stored in containers which are impervious to moisture?			
3. Do waste containers have a strength sufficient to preclude ripping, tearing, leaking, or bursting?			
4. Are waste containers labeled properly (red or orange in color with biohazard label)?			
5. Are sharps stored in rigid puncture-resistant containers?			
6. Are autoclaves routinely tested?			
7. Is infectious waste disposed of properly?			
<b>RECORDS:</b>			
Are the following written records or materials available?			
a. Employee safety training?			
b. OSHA injury and illness records?			
c. Material safety data sheets?			
d. List or inventory of hazardous chemicals?			
e. Emergency procedures?			
f. Chemical Hygiene Plan?			
g. Safety Manual?			
h. Safety inspections and assessments?			
i. Exposure monitoring data?			
j. Medical surveillance data?			
<b>MISCELLANEOUS:</b>			
1. Are broken glass containers available?			
2. Are no eating, drinking, and smoking restrictions enforced?			
3. Do employees participate in the Occupational Medical Monitoring Program?			
4. Are employees trained to know the symptoms of chemical exposure?			

**Inspected by:** \_\_\_\_\_

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

**APPENDIX B**

DIRECTORY OF SERVICE AND EMERGENCY PROVIDERS

## Directory of Service and Emergency Providers

FIU Public Safety	
Emergency	5911
Non-Emergency	
University Park Campus	(305) 348-2626
Biscayne Bay Campus	(305) 919-5555
 Environmental Health & Safety & Risk Management Services	
University Park Campus	(305) 348-2621/2622
Biscayne Bay Campus	(305) 919-5225
 Lab Safety:	
Biosafety	(305) 348-3387
Chemical Safety	(305) 348-7835
Environmental Compliance	(305) 348-2622
Radiation Safety	(305) 348-0489
 Worker's Compensation	(305) 348-7960
 University Health Services	
University Park Campus	(305) 348-2401/2402
Biscayne Bay Campus	(305) 919-5620
 Office of Human Resources	
University Park Campus	(305) 348-3273
Biscayne Bay Campus	(305) 919-5545



## **APPENDIX C**

### TABLE OF BIOSAFETY LEVELS 1-4

**TABLE 1. RECOMMENDED BIOSAFETY LEVELS FOR BIOHAZARDOUS AGENTS**

<b>BSL</b>	<b>AGENTS</b>	<b>PRACTICES</b>	<b>SAFETY EQUIPMENT (PRIMARY BARRIERS)</b>	<b>FACILITIES (SECONDARY BARRIERS)</b>
<b>1</b>	Not known to consistently cause disease in healthy adults	Standard Microbiological Practices	None Required	Open bench top sink required
<b>2</b>	Associated with human disease, hazard: percutaneous injury, ingestion, mucous membrane exposure	BSL-1 practices plus:  Limited access; Biohazard warning signs; "Sharps" precautions Biosafety manual defines waste decontamination or medical surveillance policies as needed	<i>Primary barriers:</i> Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials. <i>PPE:</i> laboratory coats, gloves, face protection as needed	BSL-1 plus: Autoclave available
<b>3</b>	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences	BSL-2 practices plus:  Controlled access; Decontamination of all waste and lab clothing before laundering; Baseline serum	<i>Primary barriers:</i> Class I or II BSCs or other physical containment devices used for all open manipulations of agents; <i>PPE:</i> protective lab clothing, gloves, respiratory protection as needed	BSL-2 plus: Physical separation from access corridors. Self-closing, double-door access. Exhausted air not recirculated. Negative airflow into laboratory
<b>4</b>	Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission	BSL-3 practices plus:  Apply PPE before entering; Shower on exit; All material decontaminated on exit from facility	<i>Primary barriers:</i> All procedures conducted in Class III BSCs or Class I or II BSCs in <u>combination with</u> full-body, air-supplied, positive pressure personnel suit	BSL-3 plus: Separate building or isolated zone Dedicated supply and exhaust, vacuum, and decon systems. Other requirements outlined in BMBL.

## GLOSSARY

**Aerosol:** A small (less than one micron) particle of solid or liquid matter that can remain suspended in the air.

**Autoclave:** A strong, pressurized, steam-heated vessel, as for laboratory experiments, sterilization, or cooking.

**Biohazardous Material:** Hazardous biological materials and organisms, including: a) infectious organisms (bacteria, fungi, parasites, prions, rickettsias, viruses, etc.) which can cause disease in healthy humans and/or significant environmental or agricultural impact; b) human or primate tissues, fluids, cells, or cell culture; c) recombinant DNA; and d) animals known to be vectors of zoonotic diseases.

**Biosafety Cabinet:** engineered, enclosed containment device designed to remove or minimize exposures to biohazardous agents.

**Biosafety Level:** four biosafety levels described consisting of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities which are appropriate for the operations performed, the infectious agent being utilized, and the laboratory function or activity.

**Decontamination:** Routinely required step of destroying/inactivating microorganisms in microbiological laboratories to protect laboratory workers and prevent contamination of their work. This is the destruction or removal of microorganisms to some lower level, but not necessarily total destruction. Sterilization, disinfection and antisepsis are all forms of decontamination.

**Disinfection:** a process that destroys the vegetative forms of an organism

**Engineering Controls:** combinations of safety equipment and facility design used to remove or minimize exposures to biohazardous agents

**Personal Protective Equipment:** equipment worn by a person to block the portals of entry of toxic materials to the body. PPE may include, but is not limited to, gloves, goggles, gowns, faceshields, and respirators.

**Standard Operating Procedures:** written instructions setting forth methods to be used to protect the health and safety of persons using biohazardous agents.

**Sterilization:** a process that destroys all living forms of an organism.

### RESOURCES

#### FIU ENVIRONMENTAL HEALTH AND SAFETY GUIDELINES

The following information can be obtained from the EH&S website at <http://ehs.fiu.edu>:

##### *Publications:*

- FIU Laboratory Safety Manual
- FIU Bloodborne Pathogen Exposure Control Plan
- FIU Radiation Safety Manual
- FIU Biomedical Waste Plan
- FIU Biosafety Cabinet Manual

##### *University Safety Compliance Guides:*

- USCG #101 Blood-borne Pathogen Exposure
- USCG #103 Control of Biohazards
- USCG #304 Infectious Waste
- USCG #604 Research Animal Care and Welfare
- USCG #907 Autoclave Safety

##### *Compliance Committees:*

- Institutional Animal Care and Use Committee
- Institutional Biosafety Committee

#### REGULATORY REQUIREMENTS AND GUIDELINES

- Florida Administrative Code, Chapter 64E-16 Biomedical Waste
- OSHA Bloodborne Pathogen Standard 29 CFR 1910.1030
- CDC Publication: Primary Containment for Biohazards: Selection, Installation, and Use of Biological Safety Cabinets
- CDC/NIH publication: Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition
- Animal Welfare Act

#### GOVERNMENT AGENCIES

- Center for Disease Control (CDC): [www.cdc.gov](http://www.cdc.gov)
- National Institute of Health (NIH): [www.nih.gov](http://www.nih.gov)
- Department of Human and Health Services (DHHS): [www.hhs.gov](http://www.hhs.gov)
- Occupational Safety and Health Association (OSHA): [www.osha.gov](http://www.osha.gov)
- World Health Organization (WHO): [www.who.int/en/](http://www.who.int/en/)
- United States Department of Agriculture (USDA): [www.usda.gov](http://www.usda.gov)
- Animal and Plant Health Inspection Services (APHIS): [www.APHIS.USDA.gov](http://www.APHIS.USDA.gov)

#### BIOSAFETY ASSOCIATED ORGANIZATIONS

- Institutional Animal Care and Use Committee (IACUC): [www.iacuc.org](http://www.iacuc.org)

- American Association for Laboratory Animal Science (AALAS):  
<http://www.aalas.org/index.aspx>