

Rice University  
Biosafety Manual



Environmental Health and Safety  
MS 123  
P.O. Box 1892  
Houston, TX 77251-1892

September 2015

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## Laboratory Biosafety Manual

### I. Introduction

This manual is intended to be a resource for information, guidelines, policies, and procedures that will enable and encourage those working in the laboratory environment to work safely and reduce or eliminate the potential for exposure to biological hazards. Most of the information in this section is taken from the book *Biosafety in Microbiological and Biomedical Laboratories* (U.S. Health and Human Services Publication No. (CDC) 21-1112, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health, 5th edition, December 2009) as well as the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (NIH Guidelines November 2013). It is intended that laboratory management will supplement this information with instruction and guidance regarding specific practices and procedures unique to the work being done in their labs.

### II. Scope

This manual is applicable to all laboratory, research, service and support activities that may involve exposure to biological and biohazardous agents or materials.

Activities which are specifically addressed are those involving:

- Viral, bacterial, fungal, and parasitic pathogens
- Recombinant DNA (rDNA)
- Biohazardous proteins and toxins
- Exposure to research animals containing a biological hazards
- Human blood, tissues, and samples
- Receipt, handling, and disposal of biological materials
- Otherwise potentially infectious material (OPIM)

### III. Containment Methods

The term containment is used to describe safe methods for managing biohazardous materials in the laboratory environment. The purpose of containment is to reduce exposure of laboratory workers or those directly handling the materials (primary containment) as well as other community members and the environment (secondary containment). The elements of containment are laboratory practice, technique, safety equipment, and facility design.

#### 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 materials must be aware of potential hazards and trained in proficient practices and techniques required for safely handling such material. When standard laboratory practices are not sufficient to control the hazard associated with a particular agent or laboratory procedure, additional measures may be needed involving safety equipment and facility design.

## Safety Equipment (Primary Barriers)

Safety equipment includes biological safety cabinets, enclosed containers, and other engineering controls designed to prevent or minimize exposures to hazardous biological materials. The use of vaccines may in some cases provide an increased level of personal protection.

## Biological Safety Cabinets (BSCs)

The biological safety cabinet is the principal device used to provide containment of biohazardous splashes or aerosols. When properly maintained and operated, they effectively contain and capture microbial contaminants and infectious agents using HEPA (High Efficiency Particulate Air) filters.

There are three types of biological safety cabinets: Class I, Class II, and Class III.

- a. Class I is an open-fronted, negative-pressure, vented cabinet with HEPA filtered exhaust. It may be equipped with a front closure and gloves for use as a glove box. The inward face velocity is a minimum of 75 linear feet per minute. Suitable for work with low-or moderate-risk biological agents, it provides protection for personnel and the environment but not for the product.
- b. Class II cabinets are open-fronted laminar-flow cabinets with a minimum inward face velocity of 75 linear feet per minute. Class II design resembles that of a fume hood but with HEPA-filtered, re-circulated mass airflow within the workspace. Exhaust air is also filtered. Class II cabinets provide protection for personnel, product, and the environment. They are designed for work with low-or moderate-risk biological agents.
- c. Class III cabinets provide the highest level of protection. Class III is a totally enclosed glove-box cabinet of gas-tight construction. The cabinet is maintained under negative air pressure of at least 0.5 inches of water gauge. Supply air is drawn into the cabinet through HEPA filters, and the exhaust air is filtered by two HEPA filters in series before it is discharged to the outside. Generally, the ventilation system is separate from the facility's ventilation system. Class III cabinets are suitable for high-risk biological agents.

Biological safety cabinets used to protect workers from hazardous biological agents must be tested and certified after installation and before use, any time they are moved, and at least annually. For more detailed information about BSCs, refer to the 5<sup>th</sup> edition of the BMBL Appendix A.

## Guidelines for the Safe Use of Class II Biological Safety Cabinets

A Class II biological safety cabinet, combined with proper microbiological technique, provides primary containment for low to moderate risk microorganisms. This containment is accomplished by laminar air flow and HEPA (high efficiency particulate air) filtration. The Class II biological safety cabinet provides protection to the product, the worker and the environment.

Class I and III cabinets are omitted from further discussion because there are none on Rice University premises. The biological safety cabinet should not be considered as a substitute for good laboratory practice. All appropriate precautions must be taken and the Class II cabinet must be used properly, as per the manufacturer's instruction as well as the CDC/NIH guidelines found in Appendix A of the BMBL. Consider that aerosols can escape. Chemical vapors will pass through HEPA filters and most biological safety cabinets do not provide protection from toxic chemicals or radionuclides. If your research requires the use of toxic materials, chemicals that are bioactive in minute quantities, or hazardous chemicals such as chemotherapeutic agents, then you must use a class II B biosafety cabinet. Class II B cabinets are ducted, and exhaust 70% or 100% of the air in the cabinet. This feature protects the user from exposure to the chemicals. Keep in mind that Class II B cabinets are only intended to be used in conjunction with small quantities of chemicals, they should not be used in the place of a fume hood.

### Preparations

1. The biological safety cabinet should be left on at all times. If the unit can not be left running continuously, turn the blower on and air purge for at least five minutes to remove airborne contamination before the next use. If the biological safety cabinet is vented to the outside of the building, both the remote motor and the internal blower must be left on at all times.
2. Turn off the UV light. Never work in the unit with the UV light illuminated. (UV light will damage the human eye very quickly).
3. The work surface should be wiped down with the appropriate disinfectant; 70% alcohol is usually suitable (if a burner is present, make sure it is not lighted), but a more appropriate disinfectant might be necessary if there is a potential for exposure to biohazards resistant to destruction by alcohol. Do not depend on the UV germicidal lamp to provide a sterile work surface. Place everything needed to complete the particular procedure inside the cabinet prior to beginning work. Remove unnecessary items; excessive materials may disrupt the air flow. Arrange implements in a logical manner to segregate clean and dirty materials.
4. Remember to provide a container for waste on the inside of the cabinet. Nothing should pass in or out through the air barrier until the procedure is complete.
5. Remove any items on the intake grilles that may block or disrupt the air supply.
6. Try to restrict the opening and closing of lab doors and walking traffic in the work area when the cabinet is being used. These activities will disturb the cabinet's air flow.

### Use of the Cabinet

1. Always wear a lab coat and gloves.
2. Conduct your work at least 4" back from the glass view panel. The middle third of the work surface is the ideal area to be used.
3. Limit arm movements and do not make fast, pumping motions. If a burner is required, use the "Touch-O-Matic" type with a pilot light. (Since a burner will produce air turbulence, place it to the rear of the workspace.) Most procedures should not require use of a flame when combined with good aseptic technique and proper cabinet use. Place a disinfectant-soaked towel on the work surface to contain any splatters or small spills that

may occur during the procedure. Do not use flammable solvents in a biological safety cabinet (disinfecting with small amounts of 70% ethanol is acceptable).

4. Control tissues, needle packages and other small loose paper or plastic products which may be caught in the air stream and pulled to the motor or HEPA filter.

### **Completion of a Job**

1. Decontaminate the surface or enclose any items which have been in direct contact with the agent.
2. Cover waste containers, and remove the waste.
3. Allow the cabinet to operate for five minutes with no activity in order to purge airborne contaminants from the work area.
4. Remove all equipment from the cabinet.
5. Decontaminate interior work surfaces. If desired, the UV light may be turned on.
6. Thoroughly wash your hands and arms with warm, soapy water.

### **Biohazardous Spills in the Cabinet**

1. Perform decontamination steps while the cabinet is operating to prevent the escape of contaminants.
2. Spray or wipe all potentially contaminated walls, work surfaces, and instruments with an appropriate disinfectant. (Make sure to wear gloves and other appropriate PPE while doing this.)
3. If the spill is large, flood the work surface with disinfectant and allow to stand 10 to 15 minutes before absorbing and wiping clean.

### **Vertical Laminar Flow "Clean Bench"**

Vertical laminar flow clean benches are not BSCs. They discharge HEPA-filtered air down onto 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 potentially infectious materials. The worker can be exposed to materials (including proteinaceous antigens) being manipulated on the clean bench, which may cause hypersensitivity. Clean air benches should never be used as a substitute for a biological safety cabinet in research, biomedical or veterinary laboratories and/or applications.

### **Other Safety Equipment**

Other safety equipment includes enclosed containers such as a safety centrifuge cup, designed to prevent release of aerosols during centrifugation. Safety equipment also includes personal protective clothing and equipment such as gloves, coats, gowns, shoe covers, boots, respirators, face masks or shields, and safety glasses or goggles. This clothing and equipment is generally used in combination with biological safety cabinets and other devices that contain the potentially hazardous agents, animals, or materials in use. If it is impractical to work in a biological safety cabinets, personal protective devices may form the primary barrier between personnel and the

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

### **Facility Design (Secondary Barriers)**

Secondary barriers protect the environment, both inside the facility and outside, as well as the community at large from biohazards and the deleterious effects associated with the uncontrolled release of biological materials. The design of the facility provides the secondary barrier. The three facility designs are the basic laboratory, the containment laboratory, and the maximum containment laboratory. The basic laboratory provides general space where work is done with viable agents that are not associated with disease in healthy adults; it includes Biosafety Levels 1. This laboratory is also appropriate for work with infectious agents or potentially infectious materials when the hazard levels are low and laboratory personnel can be adequately protected by standard laboratory practice, Biosafety Level 2. While work is commonly conducted on the open bench, certain operations are confined to biological safety cabinets. Conventional laboratory designs are adequate.

## **IV. Biosafety Levels**

The following guidelines are recommended by the Centers for Disease Control and Prevention and the National Institutes of Health and have been adopted as required procedure at Rice University. They are drawn from the manual *Biosafety in Microbiological and Biomedical Laboratories* (BMBL). The descriptions of Biosafety Levels 1-4 should be considered in conjunction with the *NIH Guidelines for Research Involving Recombinant DNA* (Risk Groups 1-4). Experience has demonstrated the prudence of the Biosafety Level 1-4 practices, procedures, and facilities described for manipulations of etiologic agents in laboratory settings and animal facilities. Strict adherence to these guidelines does contribute to a healthier and safer work environment for laboratory workers, their co-workers, and the surrounding community. To further reduce the potential for laboratory-associated infections, the guidelines presented here should be considered minimal guidance for containment. They must be customized for each individual laboratory and can be used in conjunction with other available scientific information. Four biosafety levels (BSLs) are described in Section IV of the BMBL, 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 the laboratory functions or activity. Currently, only BSL 1 & 2 agents are being used at Rice University, therefore, only these guidelines will be described here. Information on handling BSL 3 & 4 agents can be found in section IV of the BMBL.

### **Biosafety Level 1**

Biosafety Level 1 (BSL-1) is suitable for work involving agents of no known or minimal potential hazard to laboratory personnel and the environment. The laboratory may be integral to general traffic patterns in the building. Work may be conducted on open bench tops. Special containment equipment is neither required nor generally used. Laboratory personnel must have specific training in procedures conducted in the laboratory.

## Standard Microbiological Practices for BSL-1

- At the discretion of the lab supervisor, access to the laboratory must be limited or restricted to only personnel who have been fulfilled all necessary training requirements (refer to *Training Section*).
- Work surfaces must be decontaminated once a day and after any spill of viable material.
- All contaminated liquid or solid wastes shall be decontaminated before disposal. Contaminated materials that are to be decontaminated at a site outside the laboratory must be placed in a durable, leak proof, closed container before being removed from the laboratory.
- The laboratory must have an established policy for the safe handling of sharps *e.g.* needles, scalpels, pipettes, and broken glassware.
  - Needles are not bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
  - Used disposable needles and syringes are carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
  - Non-disposable sharps are placed in a hard walled container.
  - Broken glassware is not handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic-ware is substituted for glassware whenever possible.
- Mechanical pipetting devices must be used; mouth pipetting is prohibited.
- Eating, drinking, and applying cosmetics are not permitted in the work area. Food storage cabinets and refrigerators must be located outside the work area.
- Laboratory personnel must wash their hands after they handle viable materials, animals and before leaving the laboratory.
- All procedures must be performed carefully to minimize the creation of aerosols.
- An insect and rodent control program is in effect.
- Any accidents or injuries must be reported to the laboratory director or principal investigator (PI).

## Safety Equipment for BSL-1

- Special containment equipment is generally not required for manipulation of agents assigned to Biosafety Level 1.
- It is recommended that laboratory coats, gowns, or uniforms be worn to prevent contamination or soiling of personal clothing. These should either be disposable or laundered off site by a professional service.
- Laboratory personal protective equipment should never be taken home.
- Gloves should be worn if skin is broken or afflicted by a rash.
- All lab personnel wear close toed shoes that cover the entire foot, and long garment that covers the legs completely.
- Safety glasses are always worn while in the laboratory especially by persons wearing contact lenses.

## Laboratory Facilities for BSL-1

- The laboratory must be designed so that it can be easily cleaned.
- Bench tops must be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
- Laboratory furniture must be sturdy. Spaces between benches, cabinets, and equipment must be accessible for cleaning.
- Each laboratory must contain a sink for hand washing.
- All laboratories must have a door sign created with the Rice University EHS door sign generator. That accurately lists the hazards currently found in the lab and has multiple up to date contacts. This sign should be reviewed annually.
- Laboratory has doors that are self-closing and lockable for access control.
- Laboratory windows that open to the exterior are fitted with screens. An insect and rodent control program is also in effect.
- Eyewash station is readily available.

## Decontamination and Waste Disposal BSL-1

- Materials to be decontaminated offsite must be placed in a Regulated Medical Waste (RMW) container with a red liner provided by EHS.
- All cultures, stocks, and other potentially infectious materials must be decontaminated before disposal using approved disposal methods listed here in.
- All laboratory surfaces and furniture must be made of a material that is impervious to water, non-porous and can be effectively decontaminated using an appropriate disinfectant.
- Sharps containers must be sealed when  $\frac{3}{4}$  full and placed in the RMW container with a red liner for disposal.
- All biological waste, including aspirators, must be stored in a secondary container.
- The laboratory must have a spill response plan.

## Training Requirements BSL-1

- All lab personnel must attend general lab safety training provided by Rice EHS within the last year.
- All lab personnel working with nonexempt rDNA must attend **Biosafety/Bloodborne Pathogens** training provided by Rice EHS within the last year.
- The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, potential hazards present in the laboratory, and exposure evaluation procedures. This training must be documented, including general site specific training and biological site specific training.
- All personnel must receive additional training when new hazards are introduced to the lab.

## 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 are specifically trained to handle pathogenic agents and are directed by scientists who are experienced in working with these agents, (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 that may result in the creation of infectious aerosols or splashes are conducted in biological safety cabinets or other physical containment equipment. The following standards, special practices, safety equipment, and facilities apply to agents assigned to Biosafety Level 2.

### Standard Microbiological Practices for BSL-2

- At the discretion of the lab supervisor, access to the laboratory must be limited or restricted to only personnel who have been fulfilled all necessary training requirements (refer to *Training Section*).
- Work surfaces must be decontaminated once a day and after any spill of viable material.
- All contaminated liquid or solid wastes must be decontaminated before disposal. Contaminated materials that are to be decontaminated at a site outside the laboratory must be placed in a durable, leak proof, closed container before being removed from the laboratory.
- The laboratory must have an established policy for the safe handling of sharps *e.g.* needles, scalpels, pipettes, and broken glassware.
  - Needles are not bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
  - Used disposable needles and syringes are carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
  - Non-disposable sharps are placed in a hard walled container.
  - Broken glassware is not handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic-ware is substituted for glassware whenever possible.
- Mechanical pipetting devices must be used; mouth pipetting is prohibited.
- Eating, drinking, and applying cosmetics are not permitted in the work area. Food storage cabinets and refrigerators must be located outside the work area.
- Laboratory personnel must wash their hands after they handle viable materials, animals and before leaving the laboratory.
- Always remove all personal protective equipment and/or contaminated clothing before exiting the laboratory.
- All procedures must be performed carefully to minimize the creation of aerosols.
- An insect and rodent control program is in effect.
- Any accidents or injuries must be reported to the laboratory director or principal investigator (PI).

## Decontamination and Waste Disposal BSL-2

- Materials to be decontaminated offsite must be placed in a Regulated Medical Waste (RMW) container with a red liner provided by EHS.
- All cultures, stocks, and other potentially infectious materials must be decontaminated before disposal using approved disposal methods listed here in.
- All laboratory surfaces and furniture must be made of a material that is impervious to water, non-porous and can be effectively decontaminated using an appropriate disinfectant.
- Sharps containers are sealed when  $\frac{3}{4}$  full and placed in the RMW container with a red liner for disposal.
- All biological waste, including aspirators, must be stored in a secondary container.
- The laboratory should have a spill response plan.

## Training Requirements BSL-2

- All lab personnel must attend general lab safety training provided by Rice EHS within the last year.
- All lab personnel working with nonexempt rDNA must attend **Biosafety/Bloodborne Pathogens** training provided by Rice EHS within the last year.
- The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, potential hazards present in the laboratory, and exposure evaluation procedures. This training must be documented, including general site specific training and biological site specific training.
- All personnel must receive additional training when new hazards are introduced to the lab.

## Special Practices for BSL-2 Laboratories

- A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
- The laboratory must have a spill response plan.
- Access to the laboratory is limited or restricted by the supervisor when work with infectious agents is in progress. In general, persons at increased risk of acquiring infection or for whom infection may be unusually hazardous are not allowed in the laboratory or animal rooms. Persons who are immunocompromised or immunosuppressed may be at unusual risk of acquiring infections.
- The lab supervisor establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet specific requirements (e.g., immunization) enter the laboratory or animal rooms.
- Animals and plants not intended for research are not present in BSL-2 laboratories.
- When an infectious agent requires special provisions (e.g., immunization) for entering a laboratory where it is in use, a hazard warning sign incorporating the universal biohazard symbol is posted on the access door to the laboratory work area. The hazard warning sign

identifies the infectious agent, and indicates the special requirements for entering the laboratory.

- High concentrations or large volumes of infectious agents may only be centrifuged in an open lab in sealed rotor heads or safety cups.
- Laboratory personnel receive appropriate immunizations for the agents handled or potentially present in the laboratory.
- When appropriate, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional specimens may be collected periodically.
- Laboratory personnel are advised of special hazards and are **REQUIRED** to read and 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 retraining and receive additional training when procedures or policies change.
- 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 should be used in the laboratory only when there is no alternative, such as when parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles are conducted. Plastic ware should be substituted for glassware whenever possible.
- Only needle-locking syringes or disposable syringe-needle units (i.e. the 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.
- 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, and 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 as well as after work with infectious material is finished and, especially, after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated before it is sent for repair or maintenance or packaged for transport.
- Spills or accidents that result in overt exposures to infectious materials are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate at no cost and written records are maintained.

### **Safety Equipment for BSL-2**

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

1. 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 in which internal pressure may differ from ambient pressure, inoculating animals intra-nasally, and harvesting infected tissues from animals or eggs.
  2. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed 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, face shield, 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 biological safety cabinet.
  - Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn in the laboratory. This protective clothing is removed and left in the laboratory before lab personnel leave for non-laboratory areas (e.g., cafeteria, library, or offices). All protective clothing is either disposed of in the laboratory or sent to the laundry service (only after being decontaminated). It is never taken home.
  - Lab personnel wear gloves when handling infected animals and when hands may come in contact with infectious materials or contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate; if a spill or splatter occurs; the hand will be protected after the contaminated glove is removed. Gloves are disposed of when contaminated, removed when work with infectious materials is completed, and not worn outside the laboratory. Disposable gloves are not washed or reused.

### **Laboratory Facilities (Secondary Barriers) for BSL-2**

- All laboratories must have a door sign created with the Rice University EHS door sign generator. That accurately lists the hazards currently found in the lab and has multiple up to date contacts. This sign should be reviewed annually.
- A sign incorporating the universal biohazard symbol and the name of any infectious agents present is posted at the entrance to the laboratory.
- A biohazard symbol is posted on all equipment i.e., refrigerators, centrifuges, incubators, etc. that store and/or are used in the manipulation of biohazardous agents.
- Laboratory has doors that are self-closing and lockable for access control.
- Laboratory windows that open to the exterior are fitted with screens. An insect and rodent control program is also in effect.
- Eyewash station is readily available.
- Provide lockable doors for facilities that house restricted agents
- Each laboratory contains a sink for hand washing.
- The laboratory is designed so that it can be easily cleaned. Rugs are not appropriate in laboratories.
- Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

- Laboratory furniture is sturdy, and spaces between benches, cabinets, and equipment are accessible for cleaning.
- Biological safety cabinets must be installed in such a manner that fluctuations of the room supply and exhaust air do not cause them to operate outside their parameters for containment. Biological safety cabinets must be located 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.
- Biosafety cabinets (BSC) are used when procedures may create infectious aerosols or splashes.
- BSCs are used when working with large volumes or high concentrations of infectious materials
- Illumination must be adequate for all activities, avoiding reflections and glare that could impede vision.
- Vacuum lines are protected with in line HEPA filters and liquid disinfectant trap

## V. Biological Spills

A biological spill must be followed by prompt action to contain and clean up the spill. When a spill occurs, warn everyone in the area and call for assistance as needed. The degree of risk involved in the spill depends on:

1. The volume of material spilled
2. The potential concentration of organisms in the material spilled
3. The hazard of the organisms involved
4. The route of infection of the organisms, and
5. The diseases caused by the organisms.

Spills of biological agents can contaminate areas and lead to infection of laboratory workers. Prevention of exposure is the primary goal in spill containment and cleanup, exactly as in chemical spills. In evaluating the risks of spill response, generation of aerosols or droplets is a major consideration. If an accident generates droplets or aerosols in the laboratory room atmosphere, especially if the agent involved requires containment at Biosafety Level 2, the room should be evacuated immediately. Doors should be closed and clothing decontaminated. Call the Environmental Health and Safety (EHS) to supervise the cleanup. In general, a 30-minute wait is sufficient for the droplets to settle and aerosols to be reduced by air changes. Longer waiting periods may be imposed depending on the situation. If a spill of a biological agent requiring containment at Biosafety Level 2 occurs in a public area, evacuation of the area must be immediate. The supervisor is responsible for designating the extent of evacuation until EHS representative or emergency personnel arrive. Prevention of exposure to hazardous aerosols is of primary importance. Anyone cleaning a spill must wear personal protective equipment (for example, laboratory coat, shoe covers, gloves, and possible respiratory protection) to prevent exposure to organisms. An air-purifying negative-pressure respirator with **P-100** filter cartridges is generally adequate protection against inhalation of most biological agents, there may be exceptions. Contact the Biological Safety Officer for advice in choosing the correct respiratory protection and for information regarding the requirements that must be met to wear a respirator.

An appropriate chemical disinfectant should be chosen that is effective against the organisms involved in the spill

### **Sterilization, Disinfection, and Decontamination**

The Environmental Protection Agency recognizes the following categories of chemical germicides (a germicide is an agent that kills pathogenic organisms). The information in this section is drawn from *Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Third Edition* Approved Guideline, NCCIS Document M29-A3, Vol. 25 No. 10 (Clinical laboratory Standards Institutes, March, 2005).

#### **Definitions and information:**

1. Sterilizer or Sterilant: An agent intended to destroy all microorganisms and their spores on inanimate surfaces.
2. Disinfectant: An agent intended to destroy or irreversibly inactivate specific viruses, bacteria, or pathogenic fungi, but not necessarily their spores, on inanimate surfaces. Most disinfectants are not effective sterilizers.
3. Hospital Disinfectant: An agent shown to be effective against specific organisms such as *Staphylococcus aureus*, *Salmonella choleraesuis*, and *Pseudomonas aeruginosa*. It may also be effective against other organisms and some viruses. The labels of all commercially available hospital disinfectants contain a claim (which must be documented) of effectiveness for specific agents.
4. Antiseptic: A chemical germicide formulated for use on skin or tissue. Antiseptics should not be used as disinfectants.
5. Decontamination: A procedure that eliminates or reduces microbial contamination to a safe level with respect to the transmission of infection. Sterilization and disinfection procedures are often used for decontamination.

The OSHA Blood borne Pathogens Standard requires that all equipment and environmental and working surfaces must be cleaned and decontaminated after contact with blood or other potentially infectious materials. The standard also requires decontamination of contaminated work surfaces after completion of procedures, immediately or as soon as feasible after any overt contamination of surfaces or any spill of potentially infectious material, and at the end of the work shift if the work surface has become contaminated. All reusable equipment must be decontaminated immediately or as soon as feasible upon visible contamination. It should be emphasized that, for any infectious material, adequate pre-cleaning of surfaces is important for any disinfection or sterilization procedure. Ten minutes of exposure to a disinfectant may not be adequate to disinfect objects that have narrow channels or other areas that can harbor microorganisms.

#### **Alcohols**

Effective for killing hepatitis B virus (HBV) but are not recommended for this purpose because of their rapid evaporation and the consequent difficulty of maintaining proper contact times.

## Chlorine compounds

Probably the most widely used disinfectants in the laboratory. One can easily prepare an inexpensive, broad-spectrum disinfectant by diluting common household bleach.

- Bleach is a 5.25% sodium hypochlorite solution -this is equal to approximately 50,000 ppm of free available chlorine. This level of chlorine can be harmful to skin and eyes. Lower concentrations are effective in disinfection and are *less* hazardous for *the* worker. *The concentration* to be used is based on *your* assessment of the severity of the contamination or spill of infectious materials.
- For small spills of none biohazardous materials or for contamination on hard, smooth surfaces, a 1:100 dilution of commercial bleach is adequate. This is equivalent to 500 ppm of free chlorine. In the case of large or concentrated spills of infectious agents, a higher level of chlorine is needed to be effective in destroying the microorganisms. Use a 1:10 dilution (5,000 ppm of free chlorine) and flood the contaminated area with the solution. Alternatively, you can mix the disinfectant with the spilled material. This higher concentration is more suitable for porous surfaces that *may* harbor organisms in tiny cracks or pits.
- Make the solution fresh each day. Be aware that chlorine compounds may corrode metals, especially aluminum. While a 10% household bleach solution is a commonly used decontaminant concentration, it is probably stronger than necessary for ordinary uses. It can be extremely irritating to personnel. Therefore, the use of higher concentrations of bleach in chemical fume hoods, and the autoclaving of materials that have been treated with bleach, should be reserved for significant contamination.
- Note that bleach will react with water to form hypochlorous acid (HOCl), which will decompose to chlorine (Cl<sub>2</sub>) and hydrogen chloride (HCl). Special care should be taken when autoclaving hypochlorite solutions because the procedure can generate chlorine gas, which will corrode steel. To avoid evolution of chlorine, the hypochlorite solution should be neutralized with sodium thiosulfate prior to autoclaving.

**Formaldehyde** is a suspect carcinogen, so its use as a disinfectant is not recommended.

## Iodophors

If registered with the EPA may be effective hard-surface decontaminants when used per manufacturer's instructions, but iodophors formulated as antiseptics are not suitable for use as disinfectants.

## Peracetic (peroxyacetic) acid and hydrogen peroxide

These mixtures minimize the negative effects of corrosiveness sometimes seen with chlorine compounds and high concentrations of peracetic acid alone. A limited number of trade-name products containing <0.1% peracetic acid and <1.0% hydrogen peroxide and registered with the EPA as sterilants/disinfectants are available. The benefit of these products is their rapid action and broad-spectrum of germicidal activity, in addition to the reduced corrosiveness.

## Quaternary ammonium compounds

Low-level disinfectants and are not recommended for spills of human blood, blood products, or other potentially infectious materials.

## Decontamination of Spills

The following procedure is recommended for decontaminating spills of agents used at BSL-2.

1. Leave the immediate area to minimize the risk of exposure due to an aerosol.
2. Inform the other laboratory occupants, and ask them to evacuate the area, room or building depending on the severity of the spill.
3. If the spill is serious enough to require the evacuation of the entire laboratory or building please contact EHS at (713) 348-4444 or RUPD at (713) 348-6000.
4. Place a sign either in the immediate area and/or the entrance to the lab informing people that there is a biohazardous spill.
5. Don Personal Protective Equipment (PPE) appropriate for the biohazard.
  - Wear gloves and a laboratory coat or gown. Heavyweight, puncture-resistant utility gloves, such as those used for housecleaning and dishwashing, are recommended.
6. Do not handle sharps with the hands. Clean up broken glass or other sharp objects with sheets of cardboard or other rigid, disposable material. If a broom and dustpan are used, they must be decontaminated later.
7. Keep in mind that vigorously sweeping material may aerosolize material.
  - Place paper towels around and on top of the spill in order to minimize the ability of the spill to spread and produce an aerosol as you are treating it.
8. Spray freshly made biocide solution or bleach (1:9, household bleach: cool tap water) on the spill area. Cover the entire area copiously.
9. Allow 20-30 minutes for the disinfectant to sterilize the biological materials.
  - It may be necessary to use a detergent and water to clean up some biological materials. Make sure to do this after you have disinfected the area.
10. If broken glass is present use tongs or a dust pan and broom to remove all the liquids, paper towels and other waste materials
11. Clean the spill site of all visible spilled material using an aqueous detergent solution (e.g., any household detergent). Absorb the bulk of the liquid to prevent dilution of the disinfectant.
12. Absorb the disinfectant or allow it to dry.
13. Rinse the spill site with water.
14. Dispose of all contaminated materials properly. Place them in a biohazard bag or other leak proof, labeled biohazard container for sterilization.

## Biological Spill in the Open Laboratory

For a spill in the open laboratory outside a biological safety cabinet, the spill response depends on the size of the spill and hazard of the material. A minimally hazardous material spilled without generating appreciable aerosols can be cleaned with a paper towel soaked in a chemical disinfectant. A spill of a larger volume of hazardous material with aerosol generation requires evacuating the room, waiting for aerosol reduction, donning personal protective gear (including appropriate respiratory protection), selecting a disinfectant effective against the organisms involved, and cleaning as described above. Following cleanup, response personnel must wash or shower with a disinfectant soap.

## Biological Spill within a Biological Safety Cabinet

A spill that is confined within a biological safety cabinet generally presents little or no hazard to personnel in the area. Chemical disinfection procedures are to be initiated at once while the cabinet continues to operate. The disinfectant must active against the organisms of potential hazard. Flammable liquids, such as ethanol or isopropanol, are not recommended. Ethanol and isopropanol may be effective, however due to the flammable nature of these solvents and volatility they pose a significant fire hazard.

1. Spray or wipe the walls, work surfaces, and equipment with the chosen disinfectant.
2. Allow the disinfectant to remain on the surface for the appropriate contact time.
3. Minimize the generation of aerosols and use sufficient disinfectant to ensure that drain pans and catch basins below the work surface contain disinfectant. The front exhaust must also be wiped and the disinfectant drained into a container.

## Biological Spill in a Centrifuge or Other Equipment

A biological spill in a centrifuge has the potential for producing large volumes of aerosols. If a spill may have occurred within a centrifuge or other piece of equipment, turn off the equipment, warn others in the area, notify the principal investigator, allow aerosols to settle, and decontaminate following the principles described above.

## Biological Spill on a Person

If a biological material is spilled on a person, emergency response is based on the hazard of the biological agent in question, the amount of material spilled, and whether significant aerosols were generated. If aerosol formation is believed to have been associated with the spill, a contaminated person must leave the contaminated area immediately, to one that provides appropriate secondary containment for that biohazard. Avoid entering or traversing through areas not designated for research *e.g.* hallways, bathrooms.

1. If possible, they should go to another laboratory area so that hallways and other public areas do not become contaminated.
2. Contaminated clothing and personal protective equipment must be removed before exiting the laboratory and placed in red or orange biohazard bags, regulated medical waste container, or laundering service collection bag for disinfecting.

3. Before exiting the lab make sure to flush contaminated skin with water and thoroughly wash with disinfectant soap. Showering may be appropriate, depending on the extent of the spill.

## VI. Human Blood, Blood Products, and Other Potentially Infectious Materials

In any laboratory where work involves the use of and/or exposure to human blood, other bodily fluids, or unfixed human tissue there is the danger of exposure to blood borne pathogens (disease-causing microorganisms that may be found in such material). Rice University is required to comply with the OSHA Occupational Exposure to Blood borne Pathogens. Standard found in 29 CFR 1910.1030. The requirements of the standard are covered in the *Rice University Exposure Control Plan* You should refer to the *Exposure Control Plan* if your work requires occupational exposure to any of the following human materials:

### List of Fluids and Tissues of Concern

1. Blood (human blood, human blood components, and products made from human blood)
2. Semen
3. Amniotic fluid
4. Vaginal secretions
5. Saliva (in dental procedures)
6. Cerebrospinal fluid
7. Any bodily fluid that is visibly contaminated with blood
8. Synovial fluid
9. Pleural fluid all body fluids in situations where it is difficult or impossible to differentiate between body fluids
10. Pericardial fluid
11. Peritoneal fluid
12. Any unfixed tissue or organ (other than intact skin) from a human, living or dead.
13. HIV-containing cell, tissue, or organ cultures; HIV-or HBV-containing culture medium or other solutions;
14. Blood, organs, or other tissues from experimental animals infected with HIV or HBV.

Occupational exposure means reasonably anticipated skin, eye, mucous membrane, or parenteral contact with blood or other potentially infectious materials that may result from the performance of one's duties.

## VII. Recombinant DNA Research

Research involving recombinant DNA (rDNA) must comply with the National Institutes of Health's "Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules," as published December, 2013. This section briefly describes the type of research covered under the NIH guidelines. It is the responsibility of all principal investigators engaged in rDNA research to be familiar with the NIH guidelines and submit a protocol to the IBC (Institutional Biosafety Committee) prior to initiating work. Find out more information from the Rice University office of sponsored research.

- (i) molecules that a) are constructed by joining nucleic acid molecules and b) that can replicate in a living cell, i.e., recombinant nucleic acids;
- (ii) nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, i.e., synthetic nucleic acids, or
- (iii) molecules that result from the replication of those described in (i) or (ii) above.

**Section III-A**

Experiments that require Institutional Biosafety Committee approval, Recombinant DNA Advisory Committee (RAC) review, and NIH Director approval before initiation

**Section III-B**

Experiments that require NIH/ORDA and Institutional Biosafety Committee approval before initiation

**Section III-C**

Experiments that require Institutional Biosafety Committee and Institutional Review Board approval and NIH/ORDA registration before initiation

**Section III-D**

Experiments that require Institutional Biosafety Committee approval before initiation

**Section III-E**

Experiments that require Institutional Biosafety Committee notice simultaneous with initiation

**Section III-F Exempt experiments**

If an experiment falls into section III-A, III-B, or III-C and one of the other sections as well, the rules pertaining to section III-A, III-B, or III-C must be followed. If an experiment falls into section III-F alone, or into section III-F and into section 111-0 or III-E as well, the experiment is considered exempt from the NIH guidelines.

In general, the containment practices to be used for recombinant DNA research must follow those described for Biosafety Levels 1 and 2 in the CDC-NIH *Biosafety in Microbiological and Biomedical Laboratories*. However, the NIH Recombinant DNA guidelines take precedence.

**VIII. Animal Studies**

When research involves exposure to and handling of animals, there are considerations that must be given to the potential allergens, zoonosis, and physical hazards, e.g., bites and scratches, that may be encountered by researchers and staff. In general, practices for Animal Biosafety Levels 1 and 2 presented in the CDC-NIH manual, *Biosafety in Microbiological and Biomedical Laboratories*, are followed.

## IX. Management of Biological Waste

The purpose of this section is to provide information, requirements, guidelines and procedures for the handling and disposal of hazardous and non-hazardous biological waste at Rice University. In Texas, the disposal of biohazardous waste is regulated by the Texas Commission on Environmental Quality, City of Houston and the landfill disposal site.

**Biological waste:** discarded biological material from teaching, clinical, research laboratories and operations that may be composed of biological or biohazardous materials. This does not include household or office trash, waste from Food Services, Physical Plant, bedding and manure from normal agricultural operations or bedding and litter from noninfectious animals.

**Biohazardous Waste:** any solid or liquid biological waste that is hazardous because of its biological nature. All waste that contains infectious material or which, because of its biological nature, may be harmful to humans, animals, plants or the environment is biohazardous waste. This includes: waste from infectious animals; bulk human blood or blood products; microbiological waste; pathological waste; sharps; and hazardous products of recombinant DNA biotechnology and genetic manipulation.

Treatment of all laboratory biological waste prior to disposal is good laboratory practice, and is highly recommended, but biohazardous waste must be treated prior to disposal. Acceptable treatment methods include thermal or chemical disinfection, encapsulation (solidification), or incineration. The key requirements for disposal of biohazardous waste are that it must be:

1. segregated from other waste
2. securely packaged
3. specifically labeled to indicate the method of treatment
4. transported to the point of treatment or disposal by appropriately trained personnel
5. treated to eliminate the biological hazard
6. documented by maintenance of appropriate records

Biohazardous waste that is mixed with hazardous chemical waste, radioactive waste, or both must be treated to eliminate the biohazard prior to disposal. After treatment, the waste must be managed as hazardous chemical waste.

'Biohazardous Waste may also be called "medical waste", "special waste", "regulated waste", "regulated medical waste", "red bag waste", "infectious waste", or "pathological waste." For simplicity, the present document will refer to all such material as "Biohazardous Waste". Definitions in this document are derived from Title 25, Texas Administrative Code, Chapter 1.

### Segregation

Any waste that could produce laceration or puncture injuries must be disposed of as "sharps". Sharps must be segregated from other waste. Metal sharps, pasture pipettes and capillary tubes may be commingled in an approved plastic sharps container, but not with non-sharp waste. Biological waste must not be commingled with chemical waste or other laboratory trash.

## Containers

1. Containers must: be appropriate for the contents; not leak; be properly labeled; and maintain their integrity if chemical or thermal treatment is used. Containers of biohazardous material should be kept closed.
2. Metal Sharps -Must be disposed of in a commercially available sharps container with safety interlock. EHS provides sharps containers in the main office, Space Science 103 and the BRC VWR stockroom, if other sizes are required laboratories can furnish them from a vendor of their choosing.
3. Pasteur Pipets and Broken Glassware -Use a rigid, puncture-resistant container (e.g., plastic, heavy cardboard or metal) that can be sealed.
4. Solid Biohazardous Waste --Use heavy-duty plastic "Biohazard Bags" (autoclave bags) or containers for solid biohazardous waste.
5. Liquids -Use leak-proof containers able to withstand thermal or chemical treatment.

## Storage

Biohazardous waste should be treated and disposed of promptly and not allowed to accumulate. Containers holding biohazardous material must be clearly labeled, including the Biohazard Symbol. Temporary holding areas for biohazardous waste must be clean and orderly with no access to unauthorized persons (warning signs should be posted).

## Labeling Biohazardous Waste Containers

Each container of untreated biohazardous waste must be clearly identified as such and must be labeled with the Biohazard Symbol and the word "biohazards". Lab name and phone extension should be written on the exterior of the box. Label autoclave bags with commercially available autoclave tape that changes color upon thermal treatment. Apply this tape across the Biohazard Symbol on the bag before autoclaving.

## Handling and Transport

Biological waste, and biohazardous waste must only be transported and handled by personnel who have met all the training requirements for the laboratory biosafety level. Waste should only be transported on the Rice University campus or BRC campus and never between the two campuses or on public streets. Make sure that waste is always transported using a cart or dolly and that it is packaged and labeled properly. For labeling details please refer to Appendix III. Avoid transporting untreated biohazardous materials or foul or visually offensive material through non-lab or populated areas.

## X. Treatment and Disposal Methods

NOTE: Waste should be treated as near the point of origination as possible.

**Animal Carcasses, Body Parts and Solid Animal Waste (bedding, feces, etc.):** Material should be kept frozen until disposal. Contact EHS for pick up times. The Day before the pick up please place the animal remains in a red biohazard bag and package it inside one of the Regulated Medical Waste (RMW) containers provided by EHS from either the loading dock of George R. Brown Hall (GRB) or the BRC VWR stock room.

### **Sharps Disposal Procedures:**

**Sharps** - any device or object that is capable of producing lacerations or puncture injuries to the skin. Sharps can include but not limited to:

- razor blades or other cutting blades
- glass (broken or unbroken)
- glass slides
- needles
- capillary tubes

In order to prevent injury to the general public, all sharps waste must be segregated into the appropriate waste stream depending on the composition and type of contamination. Never place into trash receptacles with other general trash or paper recycling receptacles.

### **Contaminated Sharps**

Sharps used in procedures with infectious or biohazardous material are classified as regulated medical waste (RMW) and must be segregated from all other waste. All sharps waste must be placed in an approved container as specified in section IX-Containers of this document. Needles and syringes should never be disassembled or recapped prior to disposal and should be disposed of as one completed unit. Coverslips, glass slides, and ampoules may also be placed in these containers if contaminated with infectious material. Once the sharps container is  $\frac{3}{4}$  full, close and secure the container before disposing in the regulated medical waste container.

### **Noncontaminated Sharps**

Sharps waste can also be generated from other laboratories such as engineering and chemistry facilities. Although these sharps do not have the biohazardous risk of exposure, all sharps must be disposed in same sharps container with the biohazard symbol marked out or covered up. Glass pipettes and TLC plates should be disposed in the broken glass container. Once the sharps container is  $\frac{3}{4}$  full, close and secure the container before disposing in the broken glass container.

### **Radioactive Sharps**

Sharps that were used for a protocol involving radioactive materials must be handled, collected, and disposed of properly depending on the types of contamination present be it radioactive, chemical, or biological. For sharps that have radioactive contamination, use an approved sharps container with "Caution Radioactive Materials" tape placed on the box. Any biohazard symbols must be marked out or covered up if there is no biohazard contamination present. If multiple isotopes are used all waste including sharps must be segregated by isotope. A protocol must be

submitted to the Radiation Safety Officer before any work involving radioactive material can be performed. This protocol must include a sharps disposal procedure.

### **Uncontaminated Glass**

Uncontaminated, glass can be disposed of in a sturdy cardboard box with a plastic liner. These boxes can be obtained from the chemistry stockroom on campus, and from the VWR stockroom at the BRC. It is important that the box is not overfilled. Dispose of the box when no more than  $\frac{3}{4}$  full and the weight should not exceed 30 pounds. Tape the boxes shut and mark it with the lab room number or lab phone extension and place it with the ordinary trash. The box of glassware can now be placed in a regular trash dumpster.

### **Plastic Waste**

- Contaminated with Biohazardous Material: Disinfect by thermal or chemical treatment; place in municipal waste.
- Not Contaminated: Place in municipal waste.

### **Microbiological, biohazardous, infectious and rDNA Waste**

All biological and biohazardous waste must be treated before disposal either on or off site.

### **On-Site Treatment**

#### **Steam Sterilization (autoclaving)**

1. Place the waste in a plastic or metal autoclave bin.
2. Place a strip of autoclave tape on the bag of waste, or on the biohazard symbol if applicable, before beginning the sterilization cycle.
3. Use the following program parameters.
  - a. minimum temperature of 121°C
  - b. pressure above 15 psi
  - c. dwell time of at least 30 minutes
    - i. porous materials and larger loads may need a pre-vacuum cycle and/or increase the dwell time, temperature, and pressure.
  - d. fill in your information on the autoclave log book.
  - e. Longer exposure times may be necessary depending on the size and composition of waste.

### **Chemical Disinfection**

Use a chemical agent appropriate for your organism which is registered with the EPA as a disinfectant and in accordance with the manufacturer's instructions or immerse/combine the waste for not less than 20 minutes in:

1. Freshly prepared solution of 10% v/v household bleach and water. Bulk solutions can be diluted with bleach to achieve the 10% concentration.

2. Solution of 70% by volume isopropyl or ethyl alcohol (solid waste which has been immersed/combined with a liquid disinfectant must be thoroughly drained before disposal).

### **Disposal of Treated Biological Waste**

Biological waste must be treated in accordance with one of the acceptable methods listed above.

1. If treated waste is in a liquid form, dispose through the sanitary sewer after chemical disinfection with copious amounts of water.
  - a. If there is a chemical component to the waste it must be treated as chemical waste.
2. If treated waste is solid after treatment
  - a. Place a sticker noting that the material has been decontaminated on the autoclaved bag.
  - b. Place the waste in an opaque or black trash bag.
  - c. Tie the top and discard in the trash.
3. Make sure to deface any biohazard symbols before disposal.

### **Off-Site Treatment**

Wastes to be transported off-site for treatment must be packaged securely in regulated medical waste box (RMW) containers available at the George R Brown (GRB) or Bioscience Research Collaborative (BRC) loading docks.

### **Disposal of Untreated Biological Waste**

1. Waste container must be filled and weight no more than 40 pounds
2. The red interior liner must be hand-tied closed and the top of the box must be securely folded shut. If the waste material is frozen or had the potential to leak, you must use two red liners before folding shut.
3. Write the name of your principal investigator and phone extension on the exterior of the box.
4. Fill out the Hazardous Waste Pickup form or leave the box in the
  - a. BRC - Cold Storage Room off of the Loading Dock (ask the VWR stockroom for access)
  - b. Main Campus - GRB Loading Dock

Before beginning work with any biological materials e.g. toxins, prions, either resistant to chemical or heat sterilization/degradation please consult Rice EHS.

### **Genetic Material**

Disposal of materials containing recombinant DNA or genetically altered organisms must be consistent with applicable NIH Guidelines, in addition to complying with the requirements contained in this document.

## **XI. Training and Hazard Communication**

### **Training Requirements**

The laboratory manager or individual with primary supervisory responsibility must assure that all personnel who work with, or who may contact potentially biohazardous material are informed of the hazards and are trained in the proper procedures and equipment needed to avoid exposure, proper treatment and disposal of biohazardous wastes, and recognition of symptoms of infection or exposure.

All persons conducting research in a Rice University laboratory, where chemicals, biological materials, and/or physical hazards are present (radiological, laser or intense pulse light, industrial machinery, nanomaterials) must complete the “General Laboratory Safety” course as soon as possible after their employment or appointment begins. Additionally, upon entering the laboratory, and before initiating work they must complete site specific training for the laboratory. Attendance of the “General Laboratory Safety” course, and site specific training must be documents in the safety binder/manual. The “General Laboratory Safety” course must be completed on an annual basis. The site specific training must only be completed once and updated when new hazards arrive in the laboratory.

All persons conducting research in a laboratory that works with BSL-2 materials including; pathogenic microorganisms, viruses, human and non human primate cells, cell lines, human or primate tissues, human blood or bodily fluid, unfixed human, animal tissues, organs, potentially infectious material, or any active users listed on an approved IBC protocol for work that is not exempt by NIH guidelines as provided in Section III-F, must also annually attend the “Biosafety and Bloodborne Pathogens” course, and upon entering the laboratory, and before initiating work, must complete biological site specific training. A training checklist for biological site specific training can be found in Appendix I. Attendance of the “Biosafety and Bloodborne Pathogens” course, and biological site specific training must be documents in the safety binder/manual. The “Biosafety and Bloodborne Pathogens” course must be completed on an annual basis. The biological site specific training must only be completed once and updated when new hazards arrive in the laboratory.

All persons conducting experiments where radiological hazards exist necessitates attendance of the “Radiation Safety” course and radiological site specific training. Radiological hazards can include radioactive material (RAM) or source materials. If your research does not use any radiological hazards but is located in a RAM use area you should attend training. Attendance of the “Radiation Safety” course and radiological site specific training must be documents in the safety binder/manual. The “Radiation Safety” course must be completed on an annual basis. The radiological site specific training must only be completed once and updated when new hazards arrive in the laboratory.

All persons conducting experiments where class IIB or IV lasers are used must attend the “Laser Safety” course and laser site specific training. Attendance of the “Laser Safety” course and laser site specific training must be documents in the safety binder/manual. The “Laser Safety” course

must be completed on an annual basis. The laser site specific training must only be completed once and updated when new hazards arrive in the laboratory.

For more information regarding general laboratory safety, radiological safety and laser safety please check with your laboratory supervisor and read through the safety binder/manual or visit the EHS website at [safety.rice.edu](http://safety.rice.edu).

## **Course Descriptions**

Below one can find a brief synopsis of the safety courses offered by Rice University Environmental Health and Safety department. For more information please visit the website at [safety.rice.edu](http://safety.rice.edu).

### **General Laboratory Safety**

General Laboratory Safety training is designed to cover topics in general laboratory safety including personal protective equipment, the proper use of chemical fume hoods, hazardous chemical usage and segregation, hazard communication, proper waste segregation and disposal, proper gas cylinder use, spill response, and emergency response.

### **Biosafety and Bloodborne Pathogens**

The Biosafety and Bloodborne Pathogens course is designed to cover a broad range of biological safety concerns and familiarize research staff with the safety resources Rice University has to offer. This course covers the NIH/CDC classification of biological organisms and safety requirements for working with biohazards and recombinant DNA in research laboratories in addition to emergency procedures, waste management and spill response. It is designed to be compliant with the OSHA requirement for bloodborne pathogens annual in person training.

### **Radiation Safety**

Radiation Safety training is designed to cover topics in radiation safety including radiological theory, types of decay, detection, dosimetric calculations, potential effects on the human body, ALARA, administrative requirements, waste disposal, and emergency procedures.

### **Laser Safety**

Laser Safety training is designed to cover topics in laser safety including basics of lasers and laser light, laser hazard classes, laser beam injuries, and hazard control measures.

For class schedule and attendance certificates please visit the EHS website at [safety.rice.edu](http://safety.rice.edu).

### **Biological Hazard Communication**

All persons working around biohazardous materials or in laboratories that conduct research with biohazardous materials have a right to know that they may be exposed to biohazards. To this end

all laboratories working with biohazardous materials will be delineated with a biohazard symbol and the term biohazard. Additionally any equipment used to store, manipulate, or that could have become contaminated with biohazardous materials must have a biohazard symbol on it. The laboratory sign must contain the name of the organism or type of biohazardous materials present. Clear entry and exist requirements must be posted outside the laboratory.

As a part of the biological site specific training all persons working in laboratories conducting research with biohazardous materials must be advised of the types of hazards present and the symptoms associated with that disease were they to become exposed/infected.

The universal biohazard symbol can be found below it is usually found on a red or orange background.



For information regarding chemical hazard communication please refer the Rice University Chemical Hygiene Plan. For hazard communication regarding radiological materials or Lasers please refer to the Rice University Radiation Safety Manual, and Laser safety Manual respectively. All these material can be found on the EHS website at [safety.rice.edu](http://safety.rice.edu).

## Laboratory Specific Biological Safety Training

PI: \_\_\_\_\_ Department: \_\_\_\_\_

All persons working in biological laboratories must have site specific biological safety training before beginning work. Training must be conducted by a principal investigator (PI) or their designee.

List the biohazardous substances that may be found in the laboratory, including rDNA and its products that may pose a hazard to the health of laboratory staff, community, or if released into the environment:

List the symptoms associated with exposure to the materials listed above:

The following topics should be covered during the training. Check all that are covered.

- Aseptic technique
- Personal protective equipment (*i.e.* PPE as detailed in Biosafety Manual and BMBL)
- Activities of concern (*e.g.* sonication, centrifugation, sharps use etc.)
- Containment requirement (*e.g.* Biosafety Level 1 or 2)
- Disinfection and sterilization procedures (as detailed in Biosafety Manual and Spill Response Plan)
- Biological and biohazardous waste management
- Locations of required signs, notices, and EHS Biological Safety Manual
- Where biological material is used and stored within the lab(s) and restrictions on that use
- Review of written protocols involving biological and biohazardous materials
- Review emergency procedures (from Laboratory Safety Manual)
- Location of safety equipment (*e.g.* spill kits, spill cleanup materials, eye wash, safety shower *etc.*)
- Review incident reporting procedures

Instructor: \_\_\_\_\_ Researcher: \_\_\_\_\_

X \_\_\_\_\_ X \_\_\_\_\_

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

## Spill Response Plan

**What are the hazardous biological materials present in the laboratory?**

**The following disinfectants and contact times are appropriate for the biohazards presented by the biological materials found in the lab.** (e.g. 10% household bleach and 6% hydrogen peroxide are an acceptable disinfectant if the solution less than 48 hours old. You may substitute another product if it is compliant with OSHA bloodborne pathogens standard, and is certified to have germicidal activity on *Mycobacterium tuberculosis*, *Staphylococcus aureus*, and HBV. All commercial disinfectant must be mixed and used per manufacturers recommendations)

### **Biohazard spill response procedure and spill kit checklist**

This document is designed to inform laboratory workers of the supplies that should be available and the proper procedure for attending to spills involving biohazards.

#### **Biohazard spill kit checklist:**

1. Plastic bucket<sup>1</sup>
2. Dust pan<sup>1</sup>
3. Broom<sup>1</sup>
4. Mop/rags<sup>1</sup>
5. Disinfectant
6. Spray bottle
7. Paper Towels
8. Gloves<sup>2</sup>
9. Lab coat<sup>3</sup>
10. Safety Glasses<sup>4</sup>
11. Surgical Mask<sup>5</sup>
12. Bag<sup>6</sup>
13. Tongs (optional)

**Spill Response Procedure:**

- 1- Leave the immediate area to minimize the risk of exposure due to an aerosol.
- 2- Inform the other laboratory occupants, and ask them to evacuate the area, room or building depending on the severity of the spill.
- 3- If the spill is serious enough to require the evacuation of the entire laboratory or building please contact EHS at (713) 348-4444 or RUPD at (713) 348-6000.
- 4- Place a sign either in the immediate area and/or the entrance to the lab informing people that there is a biohazardous spill.
- 5- Don Personal Protective Equipment (PPE).
- 6- Place paper towels around and on top of the spill in order to minimize the ability of the spill to spread and produce an aerosol as you are treating it.
- 7- Spray freshly made biocide solution or bleach (1:9, household bleach: cool tap water) on the spill area. Cover the entire area copiously.
- 8- Allow 20-30 minutes for the disinfectant to sterilize the biological materials.
  - a. It may be necessary to use a detergent and water to clean up some biological materials. Make sure to do this after you have disinfected the area.
- 9- Use tongs or your dust pan and broom to remove all the liquids, paper towels and other waste materials\*.
- 10- Dispose of all the materials in the biohazard waste bag\*\*.
- 11- Use paper towels and disinfectant to clean up any residual materials.
- 12- Dispose of any contaminated PPE or cleaning materials in the biohazard bag.

**Footnotes**

- 1- Disposable buckets, dust pans, brooms and mop heads which can be thrown away after use are preferable. If you intend to reuse the dust pan, broom and/or mop soak the materials in a biocide solution for 30 minutes.
- 2- Double Glove when possible.
- 3- Use of a disposable lab coat is preferable; if you choose to use a reusable lab coat make sure to have it laundered after exposure.
- 4- Safety glasses should always be used, if there is a high possibility of creating an aerosol or you are working with highly pathogenic materials, chemical splash goggles and/or a face shield may be appropriate.
- 5- N95 respirator or HEPA face mask may be necessary if you are working with materials which pose a risk if aerosolized.
- 6- Double bag waste and if possible use a biohazard bag, autoclave bags preferable.

\*If glass or other sharp materials are present make sure to keep the biohazard bag in the plastic bucket.

\*\*If no glass or sharps are present you can place all the spill waste in a biohazard burn box and request a pickup on line.

## Biological Waste Management

Biological and biohazardous materials are routinely used in research laboratories throughout the Rice University campus. When laboratories have completed their investigation those materials must be disposed of according to federal, state, and local guidelines. The Texas Commission on Environmental Quality (TCEQ) has guidelines that pertain to the generation, storage and disposal of biological and biohazardous materials. This document and procedures described within are intended to assist laboratories in the management and disposal of the biological and biohazardous materials

### Definitions

**Biological hazard (biohazard)** - An agent of biological origin that has the capacity to produce harmful effects on humans and the environment.

**Biological waste**- Unwanted solid or liquid material which may be composed of or contaminated with biological or biohazardous materials.

### Recombinant DNA (rDNA) :

- (i) molecules that a) are constructed by joining nucleic acid molecules and b) that can replicate in a living cell, i.e., recombinant nucleic acids;
- (ii) nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, i.e., synthetic nucleic acids, or
- (iii) molecules that result from the replication of those described in (i) or (ii) above.

This may include but not limited to:

- Discarded cultures and stocks of microbiological, infectious agents and associated biologicals
- Discarded cultures of specimens from medical, pathological, pharmaceutical, research, clinical, commercial, and industrial laboratories.
- Discarded live and attenuated vaccines, but excluding the empty containers thereof.
- Discarded, used disposable culture dishes.
- Discarded, used disposable devices used to transfer, inoculate, and mix culture.
- Plants and soil contaminated with organisms modified with rDNA
- Materials used for and in contact with rDNA materials.

All biological and biohazardous waste must be treated before disposal either on or off site.

### On-Site Treatment

#### Steam Sterilization (autoclaving)

1. Place the waste in a plastic or metal autoclave bin.
2. Place a strip of autoclave tape on the bag, or on the biohazard symbol if applicable, of the waste before beginning the sterilization cycle.
3. Use the following program parameters.
  - a. minimum temperature of 121°C
  - b. pressure above 15 psi
  - c. dwell time of at least 30 minutes
    - i. porous materials and larger loads may need a pre-vacuum cycle and/or increase the dwell time, temperature, and pressure.
  - d. fill in your information on the autoclave log book.

### **Chemical Disinfection**

Use a chemical agent which is registered with the EPA as a disinfectant and in accordance with the manufacturer's instructions or

Immerse/combine the waste for not less than 20 minutes in:

1. Freshly prepared solution of 10% v/v household bleach and water. Bulk solutions can be diluted with bleach to achieve the 10% concentration.
2. Solution of 70% by volume isopropyl or ethyl alcohol (solid waste which has been immersed/combined with a liquid disinfectant must be thoroughly drained before disposal).

### **Disposal of Treated Biological Waste**

Biological waste must be treated in accordance with one of the acceptable methods listed above.

1. If treated waste is in a liquid form, dispose through the sanitary sewer after chemical disinfection with copious amounts of water.
  - a. If there is a chemical component to the waste it must be treated as chemical waste.
2. If treated waste is solid after treatment
  - a. Place a sticker noting that the material has been decontaminated on the autoclaved bag.
  - b. Place the waste in an opaque or black trash bag.
  - c. Tie the top and discard in the trash.
3. Make sure to deface any biohazard symbols before disposal.

### **Off-Site Treatment**

Wastes to be transported off-site for treatment must be packaged securely in regulated medical waste box (RMW) containers available at the George R Brown (GRB) or Bioscience Research Collaborative (BRC) loading docks.

**Disposal of Untreated Biological Waste**

1. Waste container must be filled and weight no more than 40 pounds
2. The red interior liner must be hand-tied closed and the top of the box must be securely folded shut. If the waste material is frozen or had the potential to leak, you must use two red bed liners before folding shut.
3. Write the name of your principal investigator and phone extension on the exterior of the box.
4. Fill out the Hazardous Waste Pickup form or leave the box in the
  - a. BRC - Cold Storage Room off of the Loading Dock
  - b. Main Campus - GRB Loading Dock

Before beginning work with any biological materials e.g. toxins, prions, either resistant to chemical or heat sterilization/degradation please consult Rice EHS.

**Biosafety Evaluation**

Building		Room(s)	
PI		Designee	

**General Microbiological Requirements**

Laboratory Practices	Y	N	NA
All personnel wash their hands after working with potentially hazardous materials and before leaving the laboratory.			
Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not allowed in the laboratory.			
Mouth pipetting is prohibited; mechanical pipetting devices are available.			
All procedures are performed to minimize the creation of splashes and/or aerosols.			
All lab personnel wear close toed shoes that cover the entire foot, and long garment that covers the legs completely.			
Safety glasses are always worn while in the laboratory especially by persons wearing contact lenses.			
Protective laboratory coats and gowns are available.			
Gloves are worn to protect hands from exposure to hazardous materials and based on appropriate risk assessment.			
Any accidents or injuries must be reported to the laboratory director or principal investigator (PI).			

Needles and Sharps Precautions	Y	N	NA
Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware are implemented.			
Needles are not bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.			
Used disposable needles and syringes are carefully placed in conveniently located puncture-resistant containers used for sharps disposal.			
Non-disposable sharps are placed in a hard walled container.			
Broken glassware is not handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware is substituted for glassware whenever possible.			

Decontamination and waste disposal	Y	N	NA
Materials to be decontaminated offsite are placed in a Regulated Medical Waste (RMW) container with a red liner provided by EHS.			
All cultures, stocks, and other potentially infectious materials are decontaminated before disposal using approved disposal methods listed in the biosafety manual.			
Work surfaces are decontaminated after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.			
All laboratory surfaces and furniture must be made of a material that is impervious to water, non-porous and can be effectively decontaminated using an appropriate disinfectant.			
Sharps containers are sealed when $\frac{3}{4}$ full and placed in the RMW container with a red liner for disposal.			
All biological waste, including aspirators, is stored in a secondary container.			
BSCs are regularly decontaminated with an approved disinfectant listed in <i>Appendix B of the Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition</i> .			
Laboratory has a spill response plan.			

Laboratory Facilities	Y	N	NA
A sign incorporating the universal biohazard symbol and the name of any infectious agents present is posted at the entrance to the laboratory.			
A biohazard symbol is posted on all equipment i.e., refrigerators, centrifuges, incubators, etc. that store and/or are used in the manipulation of biohazardous agents.			
All laboratories are required to have a door sign created with the EHS sign generator that is kept up to date.			
Laboratory has doors that are self-closing and lockable for access control.			
Laboratory has a sink for hand washing.			
Laboratory windows that open to the exterior are fitted with screens. An insect and rodent control program is also in effect.			
Bench tops must be impervious to water and resistant to chemicals used to decontaminate the work surfaces and equipment.			
Eyewash station is readily available.			
Access to the laboratory is restricted to only personnel who have been fulfilled all necessary training requirements (refer to <i>Training Section</i> )			

Training Requirements	Y	N	NA
All lab personnel have taken general lab safety training provided by Rice EHS within the last year.			
All lab personnel working in BSL 2 labs or with nonexempt rDNA have taken <b>Biosafety/Bloodborne Pathogens</b> training provided by Rice EHS within the last year.			
The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, potential hazards present in the laboratory, and exposure evaluation procedures. This training must be documented including general site specific training and biological site specific training.			
All personnel must receive additional training when new hazards are introduced to the lab.			

## Biosafety Level 2

Biosafety Level 2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that: 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

Biosafety Level 2 Practices	Y	N	NA
The laboratory supervisor must enforce the institutional policies that control access to the laboratory.			
All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.			
Biosafety cabinets (BSC) are used when procedures may create infectious aerosols or splashes			
BSCs are used when working with large volumes or high concentrations of infectious materials			
Equipment must be decontaminated before repair, maintenance, or removal from the laboratory			
Vacuum lines are protected with in line HEPA filters and liquid disinfectant trap			
Animals and plants not intended for research are not present in BSL-2 laboratories.			
Protective lab coats are worn while working and laundered by an outside commercial contractor. Lab coats should not be worn or removed outside the laboratory and never taken home to be cleaned.			
High concentrations or large volumes of infectious agents may only be centrifuged in an open lab in sealed rotor heads or safety cups.			

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Spills and accidents that result in potential exposure to infectious materials are immediately reported to the laboratory director.			
Laboratory personnel must be provided medical surveillance, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory.			
A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.			