

# Laboratory Biosafety Manual

## 1. 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. CDC99-8395, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health, 4th edition, April 1999).

It is intended that the Department Directors will supplement this information with instruction and guidance regarding specific practices and procedures unique to the work being done in their labs.

## 2. Scope

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

Activities which are specifically addressed are those involving:

- Various bacterial, fungal, and parasitic agents
- Recombinant DNA
- Exposure to research animals containing biohazardous agents
- Human blood and tissues
- Receipt, handling, and disposal of biological materials.

## 3. Containment Methods

The term containment is used to describe safe methods for managing infectious agents in the laboratory environment. The purpose of containment is to reduce exposure of laboratory workers and others to potentially hazardous agents and to prevent their escape into the outside environment. The three elements of containment are laboratory practice and 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 infected materials shall be aware of potential hazards and shall be trained and proficient in the 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 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 infectious splashes or aerosols. When properly maintained and operated, they effectively contain and capture microbial contaminants and infectious agents using HEPA (High Efficiency Particulate Air)

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

- 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.

- 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.
- 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 shall 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 1995 CDC/NIH publication "Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets."

### **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.

The biological safety cabinet is not a substitute for good laboratory practice. Aerosols can escape. Chemical vapors will pass through HEPA filters and most biological safety cabinets do not provide protection from toxic chemicals or radionuclides.

### **Preparations**

- The biological safety cabinet should be left on at all times. If the unit is not 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 should be left on at all times.
- Turn off the UV light. Never work in the unit with the UV light illuminated. (UV light will damage the human eye very quickly).
- 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). 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.
- Remember to provide a container for wastes on the inside of the cabinet. Nothing should pass in or out through the air barrier until the procedure is complete.
- Remove any items on the intake grilles that may block or disrupt the air supply.
- 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**

- Always wear a lab coat and gloves.
- 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.
- 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).

- 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**

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

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

- Perform decontamination steps while the cabinet is operating to prevent the escape of contaminants.
- Spray or wipe all potentially contaminated walls, work surfaces, and implements with an appropriate disinfectant detergent. (Make sure to wear gloves while doing this.)
- 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. An example of an enclosed container is the safety centrifuge cap, 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 agents, animals, or materials in use.

In situations in which it is impractical to work in biological safety cabinets, personal protective devices may form the primary barrier between personnel and the infectious materials. Examples of such 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 within the facility but outside the laboratory-and the community outside the facility-from exposure to infectious 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 and 2 facilities. This laboratory is also appropriate for work with infectious agents or potentially infectious materials when the hazard levels are low and laboratory personnel can be adequately protected by standard laboratory practice. While work is commonly conducted on the open bench, certain operations are confined to biological safety cabinets. Conventional laboratory designs are adequate.

#### 4. 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 book *Biosafety in Microbiological and Biomedical Laboratories*. The descriptions of Biosafety Levels 1-4 parallel those in 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. Although no national reporting system exists for reporting laboratory-associated infections, anecdotal information suggests that 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 III 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 at <http://www.cdc.gov/od/ohs/biosftv/bmb14/bmb14toc.htm>

##### **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 shall 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 shall be limited or restricted while experiments are in progress.
- A biohazard sign shall be posted at the entrance to the laboratory whenever infectious agents are present.
- Work surfaces shall 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 shall be placed in a durable, leak proof, closed container before being removed from the laboratory.
- The laboratory shall have an established policy for the safe handling of sharps.
- Mechanical pipetting devices shall be used; mouth pipetting is prohibited.
- Eating, drinking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets and refrigerators designated and used for this purpose only. Food storage cabinets and refrigerators shall be located outside the work area.
- Laboratory personnel shall wash their hands after they handle viable materials and animals and before leaving the laboratory.
- All procedures shall be performed carefully to minimize the creation of aerosols.
- An insect and rodent control program is in effect.

### **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 street clothes.
- Gloves should be worn if skin is broken or afflicted by a rash.

### **Laboratory Facilities for BSL-1**

- The laboratory shall be designed so that it can be easily cleaned.
- Bench tops shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
- Laboratory furniture shall be sturdy. Spaces between benches, cabinets, and equipment shall be accessible for cleaning.
- Each laboratory shall contain a sink for hand washing.

### **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 standard and 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 laboratory supervisor, access to the laboratory is limited or restricted while experiments are in progress.
- Laboratory personnel are to wash their hands after they handle viable materials and animals, after removing gloves, and before leaving the laboratory.
- Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas. Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.
- The laboratory shall have an established policy for the safe handling of sharps.
- Mouth pipetting is prohibited; mechanical pipetting devices are used.
- All procedures are performed carefully to minimize the creation of splashes or aerosols.
- Work surfaces are decontaminated at least once a day and after any spill of viable material.
- All cultures, stocks, and other regulated wastes are decontaminated by an approved decontamination method, such as autoclaving before disposal. Materials to be decontaminated outside the immediate laboratory are to be placed in a durable, leak proof container that is closed for transport from the laboratory.
- An insect and rodent control program is in effect.

#### **Special Practices for BSL-2**

- 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.

- 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.
- 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 to employees, 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**

- 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.
- An eyewash facility is readily available.
- Biological safety cabinets shall 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 shall 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.
- Illumination shall be adequate for all activities, avoiding reflections and glare that could impede vision.

## **5. Biological Spills**

A biological spill shall 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:

- the volume of material spilled
- the potential concentration of organisms in the material spilled
- the hazard of the organisms involved
- the route of infection of the organisms, and
- 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 shall be evacuated immediately.

Doors shall be closed and clothing decontaminated. Call the Biological Safety Officer 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 shall be immediate. The Supervisor shall be responsible for designating the extent of evacuation until Biosafety Safety Officer or emergency personnel arrive. Prevention of exposure to hazardous aerosols is of primary importance.

Anyone cleaning a spill shall 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. However, 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 Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue*, Approved Guideline, NCCIS Document M29-A, Vol. 17, NO.20 (National Committee for Clinical Laboratory Standards, December, 1997).

- **Sterilizer or Sterilant:** An agent intended to destroy all microorganisms and their spores on inanimate surfaces.
- **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.
- **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.
- **Antiseptic:** A chemical germicide formulated for use on skin or tissue. Antiseptics should not be used as disinfectants.
- **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 shall 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 shall 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, for example, are 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** are probably the most widely used disinfectants in the laboratory. You 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 infectious agents 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. The Biological Safety Officer should be consulted prior to autoclaving any items treated with bleach.

**Formaldehyde** is an OSHA-regulated chemical that is a suspect carcinogen, so its use as a disinfectant is not recommended.

**Iodophors** that are 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** 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** are 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.

- Wear gloves and a laboratory coat or gown. Heavyweight, puncture-resistant utility gloves, such as those used for housecleaning and dishwashing, are recommended.
- 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.
- Avoid generating aerosols by sweeping.
- Absorb the spill. Most disinfectants are less effective in the presence of high concentrations of protein, so absorb the bulk of the liquid before applying disinfectants. Use disposable absorbent material such as paper towels. After absorption of the liquid, dispose of all contaminated materials as waste.
- 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.
- Disinfect the spill site using an appropriate disinfectant, such as a household bleach solution. Flood the spill site or wipe it down with disposable towels soaked in the disinfectant.
- Absorb the disinfectant or allow **it** to dry.
- Rinse the spill site with water.

- 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 shall 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. However, chemical disinfection procedures are to be initiated at once while the cabinet continues to operate. The disinfectant shall be one that is active against the organisms of potential hazard. Flammable liquids, such as ethanol or isopropanol, shall not be used, even if effective, because of the fire hazard of generating dangerous vapor concentrations within the cabinet that could be ignited by an electrical spark or other source.

Spray or wipe the walls, work surfaces, and equipment with the chosen disinfectant. Allow the disinfectant to remain on the surface for the appropriate contact time (refer to Appendix C Table 3 and 4 for recommended contact times).

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 shall 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. On becoming aware that 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 spilled, 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 shall leave the contaminated area immediately. If possible, (s)he should go to another laboratory area so that hallways and other public areas do not become contaminated.

Contaminated clothing is removed and placed in red or orange biohazard bags for disinfecting. Contaminated skin shall be flushed with water and thoroughly washed with a disinfectant soap. Showering may be appropriate, depending on the extent of the spill.

## **6. Human Blood, Blood Products, and Other Potentially Infectious Materials**

In any laboratory where work involves the use of and/or exposure to human blood, certain other body fluids, or unfixed human tissue, there is the danger of exposure to blood borne pathogens, the 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:

Blood (human blood, human blood components, and products made from human blood)

These human body fluids:

Semen

amniotic fluid

vaginal secretions

saliva (in dental procedures)

cerebrospinal fluid

any body fluid that is visibly contaminated with synovial fluid

pleural fluid all body fluids in situations where it is difficult or impossible to differentiate between body fluids

pericardial fluid

peritoneal fluid

Any unfixed tissue or organ (other than intact skin) from a human, living or dead.

HIV-containing cell, tissue, or organ cultures; HIV-or HBV-containing culture medium or other solutions;

and 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 an employee's duties.

## 7. Recombinant DNA Research

Recombinant DNA research shall comply with the National Institutes of Health's "Guidelines for Research Involving Recombinant DNA Molecules," as published in the Federal Register, July 5, 1994, Volume 59, No. 127, pages 34,496 through 34,547, and any subsequent amendments thereto (latest: Amendment Effective December 28, 2000, FR, January 5, 2001 [66 FR 1146]).

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 shall 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 shall 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.

## 8. Animal Studies

When research involves exposure to and handling of animals, there are considerations that must be given to the potential allergens, zoonoses, 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. (See <http://bmbf.od.nih.gov/sect4tab1.htm> for a good summary of the Animal Biosafety Levels).

## 9. 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 Department of Health and the Texas Commission on Environmental Quality. Local regulations of the City of Houston, and Brown and Ferris (BFI) also

apply to all waste that will be disposed in their landfills.

**BIOLOGICAL WASTE** means discarded biological material from teaching, clinical, and research laboratories and operations. 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** means any solid or liquid biological waste that is hazardous because of its physical and/or 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; and (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", "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.

Waste that is to be incinerated should not be commingled with glass or plastics.

Biological waste must not be commingled with chemical waste or other laboratory trash.

### **Containers**

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.

**Metal Sharps** --Use a rigid, puncture-resistant container (heavy-walled plastic is recommended) suitable for encapsulation and disposal. Container and encapsulated contents must withstand an applied pressure of 40 psi without rupture.

**Pasteur Pipets and Broken Glassware** -Use a rigid, puncture-resistant container (e.g., plastic, heavy cardboard or metal) that can be sealed.

**Solid Biohazardous Waste** --Use heavy-duty plastic "BIOHAZARD BAGS" (autoclave bags) or containers for solid biohazardous waste.

**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.

Each container of treated biohazardous waste is to be placed in a red plastic container provided by Stericycle, Inc.

(biohazardous waste disposal service provider).

Label autoclave bags with commercially available autoclave tape that changes color upon adequate thermal treatment. Apply this tape across the Biohazard Symbol on the bag before autoclaving.

### **Handling and Transport**

Only properly trained technical personnel can handle or transport untreated biohazardous waste.

Treated waste must also be transported by properly trained technical personnel.

Avoid transporting untreated biohazardous materials or foul or visually offensive material through non-lab or populated areas.

## **10. 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.):**

This waste is sent to a commercial plant for incineration. The landfill will not accept carcasses or recognizable body parts.

### **Metal Sharps:**

Discarded metal sharps MUST be contained and disposed of in a manner that prevents injury to laboratory, custodial and landfill workers. Needles, blades, etc., are considered BIOHAZARDOUS even if they are sterile, capped and in the original container. Never place sharps in a trash container or plastic bag that might be handled by custodial staff.

### **Pasteur Pipets and Broken glassware:**

- Contaminated with Biohazardous Materials: Disinfect by thermal or chemical treatment; place in a cardboard box. When full, tape the box shut and place near trash for custodians
- Not contaminated with Biohazardous Material: Place in a cardboard box. When full, tape the box shut and place near trash for custodians

### **Plastic Waste**

- Contaminated with Biohazardous Material: Disinfect by thermal or chemical treatment; place in a trash dumpster.
- Not Contaminated: Place in a trash can.

### **Microbiological Waste**

- Solid --Disinfect by thermal or chemical treatment; place in a trash can lined with an opaque trash bag.
- Liquid --Disinfect by thermal or chemical treatment; discharge into the sewer system.

### **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.

### **Radioactive Waste**

Biological waste that contains radioactive material must be managed as radioactive waste.

### **Chemical Waste**

Biohazardous waste which also contains hazardous chemicals must be managed as hazardous chemical waste.

## **11. Training and Hazard Communication**

The Department Directors 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.

**Written Procedures and Records**

Environmental Health and Safety will maintain written records that, at a minimum, contain the following information:

Date of treatment

Quantity of waste treated

Method/conditions of treatment

Name (printed) and initials of the person performing the treatment.