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# Biological Safety Manual

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EHS Biological Safety Staff

University of Iowa

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# Biological Safety Manual

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# Introduction

Laboratories handling biohazardous agents are special, often unique, environments that may pose an infectious disease risk to persons in or near them. Fewer than 20 percent of all cases of laboratory-acquired infections are associated with a known incident. Exposure to infectious aerosols is a plausible, but usually unconfirmed, source of infection. The knowledge, techniques and equipment needed to prevent most laboratory-acquired infections are readily available. This publication was prepared as an aid to researchers to prevent the infection of laboratory workers and ancillary personnel. It serves as the written Biological Safety Program for facilities at The University of Iowa. All personnel engaged in the use of infectious or hazardous biological (biohazardous) agents must participate in this program.

The goal of the University's Biological Safety Program is to protect staff, students and the environment from exposure to biohazardous agents, as well as the protection of experimental materials. Prior to removal from the clinical or research laboratory area, biohazardous/infectious wastes must be properly packaged and labeled for subsequent decontamination and disposal. The responsibility for identifying and disposing of biohazardous materials rests with the PI's (principal investigators) or laboratory supervisors. This responsibility cannot be shifted to inexperienced or untrained personnel.

Principal investigators or laboratory supervisors should contact the Biological Safety office if there is uncertainty about categorizing, handling, storing, treating, or discarding biologically derived material. Appropriate contact information for each biosafety program is available on the [contact us link](#) on our website. A list of additional resources containing biosafety information is located in Appendix A.

## I Abbreviations

Abbreviation	Definition
ABSA	American Biological Safety Association
ABSL	Animal Biosafety Level
APHIS	Animal and Plant Health Inspection Service
ARO	Alternate Responsible Official
BBP	Bloodborne Pathogens
BMBL	Biosafety in Microbiological and Biomedical Laboratories
BSC	Biological Safety Cabinet (aka Tissue Culture Hood/Cabinet)
BSL	Biosafety Level
BSO	Biological Safety Officer
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
DHHS	Department of Health and Human Services
DNR	Department of Natural Resources
DOT	Department of Transportation

<b>Abbreviation</b>	<b>Definition</b>
DPS	Department of Public Safety (UI Police Department)
EHS	Environmental Health & Safety Office
ETC	Emergency Treatment Center
FBI	Federal Bureau of Investigation
FM	Facilities Management
GMT	Good Microbiological Techniques
HEPA	High Efficiency Particulate Air
HHMI	Howard Hughes Medical Institute
IACUC	Institutional Animal Care and Use Committee
IATA	International Air Transport Association
IBC	Institutional Biosafety Committee
IRB	Institutional Review Board
IOSH	Iowa Occupational Safety and Health Administration
MSDS	Material Safety Data Sheet
NCI	National Cancer Institute
NIH	National Institutes of Health
NSF	National Sanitation Foundation
OSHA	Occupational Safety and Health Administration
OSP	Office of Science Policy
OPIM	Other Potentially Infectious Materials
PI	Principal Investigator
PPE	Personal Protective Equipment
ppm	parts per million
RAC	Recombinant DNA Advisory Committee
rDNA	Recombinant DNA
RG	Risk Group
RO	Responsible Official
SOP	Standard Operating Procedure
UEHC	University Employee Health Clinic
UIHC	University of Iowa Hospitals and Clinics
USDA	United States Department of Agriculture
USPS	United States Postal Service
UV	Ultra-violet
WHO	World Health Organization

## **II Program Administration**

### **a. University responsibilities**

Biological Safety will be enforced by the Iowa Occupational Safety and Health Administration (IOSH) under the General Duty Clause, referencing existing standards that include recommendations from the Centers for Disease Control and Prevention (CDC) and National Institutes of Health (NIH), for example.

The University is responsible for implementing and maintaining the Biological Safety Program in work areas. This responsibility typically rests with each department, either with a departmental safety committee or with one or more individuals selected to serve as coordinator(s).

The University is obligated to include the following elements in its biological safety program:

- A determination of the required levels of protective apparel, equipment and containment facilities and assurance that these are adequate and in working order.
  - A personal protective equipment [hazard assessment tool](#) is available from EHS that suggests the recommended PPE for several common laboratory activities.
  - Recommended levels of containment and associated facilities can be found in the [NIH Guidelines](#) (*National Institutes of Health Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*) and in the [BMBL](#) (Biosafety in Microbiological and Biomedical Laboratories).
- Appropriate training to ensure workers know and follow proper biological safety procedures. Recommended training for laboratory personnel can be found on EHS's Web site and in [Section XII](#).
- Regular biosafety inspections of facilities and equipment. Staff from EHS will annually certify all fume hoods. EHS biosafety staff will arrange for ENV Services to annually certify all biological safety cabinets (BSCs). The principal investigator (PI) is responsible for scheduling any necessary maintenance.
- Assurance of knowledge and compliance with current legal and University requirements concerning biological safety. Training material and annual laboratory self-audits (described below) will assist departments and PIs in meeting this requirement.

## **b. Employee rights and responsibilities**

### **i Employee rights**

- To be informed of the health hazards of biological agents in their work areas.
- To be properly trained to work safely with these agents.
- To be given access to CDC recommended vaccinations, as appropriate, if potentially exposed to certain biohazardous agents (e.g., vaccinia and hepatitis B).

### **ii Employee responsibilities**

- Attend training programs concerning biological safety.
- Stay informed about biohazardous agents in their work areas.
- Use work practices and protective equipment required for safe performance of their job.

- Plan and conduct each operation in accordance with recognized biological safety procedures.
- Inform their supervisors of accidents, conditions or work practices they believe to be a hazard to their health or to the health of others.

### **c. Laboratory audit**

Each PI is assigned an EHS Safety Advisor, who is available to answer any questions related to lab safety. The Safety Advisor also conducts an annual Lab Safety Audit and an unannounced walkthrough (Lab Safety Round) of the lab. The PI may designate a staff member who is familiar with the lab's research to address the audit questions on his/her behalf. Approximately two weeks prior to the audit, the PI/designated staff member will receive a notice from the respective lab Safety Advisor requesting to schedule a mutually convenient date and time for the lab visit. It is the responsibility of the PI or designated staff member to ensure compliance of regulations, and certify training of all personnel. An online [Audit Topics Checklist](#) is available, which serves as a guidance document to help prepare for the audit; this form does not need to be filled out, but lists the items that will be covered during the audit.

The audit is intended to facilitate the integration of the Biological and Chemical Safety programs at the departmental level and improve compliance status in labs. The respective Safety Advisor will visit the lab to review the items listed in the Audit Topics Checklist, and discuss any questions or concerns with the PI and/or lab staff. A walkthrough of the lab will be completed to review chemical/hazardous waste storage, BSC and fume hood certifications, biohazard sign postings, select toxin inventories, training records and other various biosafety compliance issues. The EHS Safety Advisor Team helps to educate and guide laboratory personnel in the evolving regulations and guidelines established by regulatory boards, prudent work practices and University requirements.

## **III Principles of Biosafety**

### **a. Biohazardous agents / material**

A "biohazardous agent" is generally an agent that is biological in nature, capable of self-replication and possesses the capacity to produce deleterious effects upon biological organisms. A biohazardous material is any material that contains or has been contaminated by a biohazardous agent. In addition, the University considers any material originating in medical areas, patient care, and biomedical research as biohazardous.

Biohazardous agents include, but are not limited to:

- Viruses
- Bacteria
- Rickettsia
- Parasites

- Fungi
- Recombinant and synthetic nucleic acid molecules
- Cultured animal cells (and potentially biohazardous agents they may contain)
- Human clinical specimens (tissues, fluids, etc.)
- Tissues from experimental animals (including animal dander)

Containment and safe handling of potentially biohazardous materials require strict adherence to prudent microbiological practices. Procedures and practices are outlined within [Section VI](#) and [Section VII](#). The general risk group/biosafety level designation to which organisms have been assigned can be found in the [NIH Guidelines](#) and in the [BMBL](#). In many cases, the risk group of the agent is the same as the biosafety level designation. These classifications are based on activities typically associated with the growth and manipulation of the quantities and concentrations of infectious agents required to accomplish identification or typing. Additional precautions and increased levels of primary and secondary containment may be indicated in certain situations. Areas where increased safety measures may be needed include activities involving large volumes and/or concentrated preparations ("production quantities"), activities which are likely to produce aerosols or those that are otherwise intrinsically hazardous.

When working with biohazardous materials, it is of primary importance that procedures are followed consistently. Additionally, motivation, critical judgment and specific safety knowledge regarding the material or agent are essential for ensuring the protection of all personnel, the public and the environment.

## **b. Risk assessment**

One of the most important aspects of biosafety is assessing the risk associated with the laboratory manipulations of the agent or organism under investigation. Risk assessments should be performed by individuals most familiar with the specific characteristics of the agent/organism being considered for use, the equipment and procedures to be employed, animal models that may be used and the containment equipment and facilities available. The laboratory director or PI is responsible for ensuring that adequate risk assessments are performed and that appropriate equipment and facilities are available to support the work being proposed.

### **i Risk group**

One of the most helpful tools available for performing a risk assessment is the listing of risk groups for microbiological agents. Table 1 below describes how microorganisms are classified into risk groups; the *NIH Guidelines* and the BMBL lists individual microorganisms according to their assigned risk group/biosafety designation.

However, simple reference to the risk group for a particular agent is insufficient in the conduct of a risk assessment. Other factors that should be considered, as appropriate, include:

- Pathogenicity of the agent and infectious dose
- Routes of infection (parenteral, airborne, ingestion)
- Stability of the agent in the environment
- Presence of a suitable host (human or animal)
- Laboratory activity planned (concentration, sonication, aerosolization, centrifugation, large volumes, etc.)
- Any genetic manipulation of the organism that may extend the host range of the agent or alter the agent’s sensitivity to known, effective treatment regimens
- Local availability of effective prophylaxis or therapeutic interventions

On the basis of the information ascertained during the risk assessment, a biosafety level (BSL) (see [Section VI](#)) can be assigned to the planned work and appropriate personal protective equipment selected.

**Table 1. Classification of infectious microorganisms by risk group**

<b>Risk Group Classification</b>	<b>Disease Association</b>	<b>Individual/Community Risk</b>
Risk Group 1	Microorganisms not associated with disease in healthy adult humans or animals.	No or low individual and community risk.
Risk Group 2	Microorganisms associated with human or animal disease that is rarely serious and for which preventative or therapeutic interventions are <i>often</i> available.	Moderate individual risk; low community risk.
Risk Group 3	Microorganisms associated with serious or lethal human or animal disease for which preventative or therapeutic interventions may be available.	High individual risk; low community risk.
Risk Group 4	Microorganisms likely to cause serious or lethal human or animal disease for which preventative or therapeutic interventions are not usually available.	High individual risk; high community risk.

## ii **Specimens for which there is limited information**

Situations exist when the available information is insufficient to perform an appropriate risk assessment, e.g., with clinical specimens or epidemiological samples collected in the field. In these cases, it is prudent to take a conservative approach to specimen manipulation, including:

- [Universal precautions](#)
- [Personal protective equipment](#)
- [Basic laboratory containment](#)

## c. **Routes of exposure**

Unlike chemical and physical agents where "safe" doses are specified in technical literature, no such assignment has been made to biohazardous agents. Thus, controlling exposures to these agents becomes the greatest task in the prevention of subsequent infections. In order to accomplish this goal, it is necessary to understand the routes by which exposure to these agents occurs. The most common routes are: respiratory (from the generation of aerosols); contact; ingestion; mucous membrane; and inoculation (parenteral).

### i **Generation of aerosols**

It is generally conceded that aerosols are the primary means by which infectious diseases are contracted or spread in the microbiological laboratory, although many are known to have occurred from animal bites, needlesticks, and similar situations where direct contact can occur.

Laboratory-associated infections represent an occupational hazard for all personnel working in institutions where infectious agents are handled. Of the nearly 4,000 reported laboratory associated infections, just over 4 percent proved fatal. Less than 20 percent of the infections could be attributed to some known accident, such as accidental inoculation. Although not directly proven, aerosol production could account for a large part of those accidents listed as due to "unknown causes." The majority of laboratory-associated infections occurred at institutions engaged in research or clinical diagnostic work. It is interesting to note that the personnel involved were generally trained professionals, those individuals most knowledgeable about laboratory hazards.

There are many opportunities for aerosols to be generated through normal laboratory procedures. The number of organisms sufficient to cause infections in humans can be present in a single droplet. Studies have been conducted to determine the average number of droplets created by many typical operations, and it was found that some procedures are prolific aerosol generators. Some of the laboratory operations which release a substantial number of droplets seem almost trivial in nature, such as: breaking bubbles on the surface of a culture as it is stirred; streaking a rough agar plate with a loop; using a vortex to mix a liquid and then removing the cap too soon; dispensing the last drop from a

pipette; inserting a hot loop into a culture; pulling a stopper or a cotton plug from a bottle or flask; taking a sample from a diaphragm-stoppered container; opening and closing a Petri dish; opening a lyophilized culture; etc. Most of these only take a few seconds and are often repeated many times a day. Other more complicated procedures might be considered more likely to release organisms into the air, such as grinding tissue with a mortar and pestle, conducting an autopsy on a small animal, harvesting infected tissue from animals or eggs, intranasal inoculation of small animals and opening a blender too quickly.

Some incidents have occurred by failing to take into account the possibility that accidents can happen, such as a tube breaking in a centrifuge. The possibility of aerosol production should always be considered while working with infectious organisms.

## **ii Contact**

Control of potential exposure by contact requires procedures be conducted in a manner that avoids contamination of body or work surfaces. This is accomplished through the use of gloves and other personal protective equipment, protection of work surfaces with an appropriate absorbent disposable covering, using care in the performance of procedures and cleaning and disinfecting work surfaces. Dispersal of contaminants to other surfaces can occur by their transfer from gloves of a laboratory worker, by placement of contaminated equipment or labware and by improper packaging of contaminated waste.

## **iii Ingestion**

A number of procedures carried out in the laboratory and animal facility offers the potential for either direct or indirect exposure by the oral route. The procedure that offers the greatest potential for exposure by ingestion is mouth pipetting. Clearly, such exposures are completely avoidable through the use of mechanical pipetting devices. Indirect oral exposures can be avoided by practicing good personal hygiene that includes regular hand washing and not placing any objects, including fingers, into the mouth. Wearing a surgical mask or face shield will serve as protection against splashes or splatters of biohazardous material into the mouth.

## **iv Mucous membrane**

Wearing a face shield can protect workers against splashing biohazardous material into the eyes, nose, and mouth. Safety glasses or goggles provide protection from splashes to the workers' eyes. Avoid all hand-to-eye, hand-to-nose, and hand-to-mouth contact.

## v **Inoculation**

The single procedure that presents the greatest risk of exposure through inoculation is the use of sharps; most often in the form of a needle and syringe. Inoculation can also result from animal bites and scratches.

Needle/syringe devices are used principally for the transfer of materials from diaphragm-stoppered containers and for the inoculation of animals. Their use in the transfer of materials from diaphragm-stoppered containers can result in dispersal of biohazardous material onto surfaces and into the air. Depending on the route of inoculation of animals, using a needle and syringe may also result in contamination of body surfaces. Because of the imminent hazard of self-inoculation, the use of a needle and syringe should be limited to those procedures where there is no alternative. When needles must be used, employ safety syringes and perform such procedures with the greatest possible care.

### Safe Handling and Disposal of Sharps

- Handling should be kept to a minimum
- **Never recap needles using two hands**
- Forceps or other grabbing devices should be used whenever possible
- Always place disposable sharps into a sharps container at the point of use
- Always dispose of syringes and needs as a whole unit (with the needle uncapped)
- Never overfill the sharps container

Needles should not be recapped, bent, removed or manipulated by hand. However, if it is essential that a needle be recapped due to the nature of the work, it must be done using a mechanical device or the one-handed scoop method, as follows:

1. Place the cap on a flat surface with something firm to “push” the needle cap against.
2. Holding the syringe in one hand, slip the needle into the cap without using the other hand.
3. Push the capped needle against a firm object to secure the cap firmly onto the needle.

## d. **Biohazardous / infectious waste**

In Iowa, disposal of waste is regulated by the DNR (Department of Natural Resources). The DNR defines infectious waste as waste that includes the categories listed below. Infectious means containing pathogens with sufficient virulence and quantity so that exposure to an infectious agent by a susceptible host could result in an infectious disease when the infectious agent is improperly treated, stored, transported or disposed. At the University, infectious waste and biohazardous waste are synonymous.

Since a precise definition of infectious waste, based on the quantity and type of etiologic agents present is virtually impossible, the most practical approach to infectious waste management is to identify those categories of waste that have the greatest potential for transmitting disease. The following categories of waste are designated as infectious; note, the following lists are not exhaustive.

**i Cultures and stocks**

- Specimens from medical, pathology and research laboratories
- Disposable culture/Petri dishes and devices used to transfer, inoculate, and mix cultures
- Waste from the production of biologicals
- Discarded live and attenuated vaccines

**ii Pathological wastes and human body fluids**

- Tissues, organs, body parts and body fluids that are removed during surgery, autopsy, or other medical procedures
- Specimens of body fluids and their containers
- Cerebrospinal fluid, synovial fluid, pleural fluid, peritoneal fluid, pericardial fluid, and amniotic fluid from humans

**iii Human blood and blood products**

- Waste human blood
- Products of human blood
- Items saturated and/or dripping with human blood
- Items that were saturated with human blood that are now caked with dried human blood
- Serum, plasma, and other blood components, and their container(s), which were used in either patient care, testing and laboratory analysis or the development of pharmaceuticals

**iv Sharps, needles and hypodermics**

- Sharps that have been used in animal or human patient care or treatment or in medical, research or industrial laboratories, including hypodermic needles, syringes, Pasteur pipettes, scalpel blades, razor blades and needles with attached tubing
- Broken or unbroken glassware that was in contact with infectious agents
- Used slides and cover slips
- Shards of contaminated broken glass

**v Animal carcasses and bedding**

Animal carcasses, body parts and bedding of animals that were known to have been exposed to infectious agents during research, production of biological material or testing of pharmaceuticals are infectious waste.

## **vi Isolation wastes**

These include all wastes that are biological or discarded materials contaminated with blood, excretion, exudates or secretions from humans who are isolated to protect others from highly communicable diseases.

## **vii Other laboratory wastes**

- Specimen containers
- Disposable gloves, lab coats, masks, booties and aprons
- Disposable pipettes
- All cell culture materials
- All microorganisms constructed using recombinant techniques
- Pipette tips
- Solidified blood and body fluids
- Waste that has been steam sterilized

Please refer to the [Biohazard Waste Guidelines](#) located on EHS's Web site for questions regarding biohazardous waste types and segregation, packing, and pickup of waste.

## **e. Containment**

The term containment is used to describe safe methods for managing biohazardous agents in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce exposure of laboratory workers and other persons and to prevent escape of potentially biohazardous agents into the environment. Three elements of containment are laboratory practice and technique, safety equipment, and facility design.

### **i Laboratory practices and techniques**

The most important element of containment is strict adherence to standard microbiological practices and techniques. Persons working with biohazardous agents or materials must be aware of the potential hazards in addition to being trained and proficient in the techniques required for safe handling of the material. When standard laboratory practices are not sufficient to control the hazard(s) associated with a particular agent, additional measures may be needed. Safe practices and techniques must be supplemented by appropriate facility design, engineering features, safety equipment and management practices.

Each laboratory should develop or adopt a biological safety plan that identifies the hazards that may be encountered and specifies practices and procedures designed to minimize or eliminate risk. Personnel should be advised of special hazards and should be required to follow the specified practices.

## ii **Safety equipment**

Safety equipment includes fume hoods, animal microisolator caging, biological safety cabinets (BSC), and a variety of other equipment such as enclosed containers (e.g., the safety centrifuge cup, which is designed to prevent aerosol release during centrifugation). The BSC is the principal device used to provide containment of aerosols generated by many microbiological procedures. The three types of BSCs (Class I, II, III) used in microbiological laboratories are described in [Section V.d](#). Open fronted Class I and Class II BSCs are partial containment cabinets that offer significant levels of protection to laboratory personnel and to the environment when used with good microbiological techniques. The gas-tight Class III BSC provides the highest attainable level of protection from an equipment standpoint.

Safety equipment also includes items for personal protection such as gloves, coats, gowns, shoe covers, boots, respirators, face shields and safety glasses. These personal protective devices are often used in combination with biological safety cabinets and other devices that contain the agents, animals or materials being studied. In some situations, where it is impractical to work in a BSC, personal protective equipment may form the primary barrier between personnel and biohazardous materials. Examples of such activities include certain animal studies, large animal necropsy, production activities and activities relating to maintenance, service or support of the laboratory facility.

## iii **Facility design**

Facility design is important for providing a barrier to protect persons working outside the laboratory from biohazardous agents that may be accidentally released inside the laboratory. Laboratory management is responsible for providing facilities commensurate with the laboratory's function. Described below are three facility designs by level of containment in ascending order. This is a brief description of the actual facility requirements for each level of containment. The requirements for each level are detailed in their entirety in [Section VI](#).

The basic laboratory provides general space appropriate for work with defined biohazardous agents that are not associated with disease processes in healthy adults and do not colonize in humans. All activities are regularly conducted on the open bench using standard laboratory practices.

The containment laboratory provides general space appropriate for work with biohazardous agents or potentially biohazardous materials when the hazard levels are low and laboratory personnel can be adequately protected by

standard laboratory practice. Work is commonly conducted on open benches with certain operations confined to BSCs. Conventional laboratory designs are adequate. Areas known to be sources of general contamination such as animal rooms and waste staging areas should not be adjacent to media processing areas, tissue culture laboratories or patient care activities. Public areas and general offices to which non-laboratory staff requires frequent access should be separated from space that primarily supports laboratory functions.

The high containment laboratory has special engineering features that make it possible for laboratory workers to handle hazardous materials. Unique features that distinguish this laboratory from the basic and containment laboratories are provisions for access control and a specialized ventilation system. The high containment laboratory may be an entire building or a single module or complex of modules within a building. In all cases, the laboratory is separated from areas open to the public by a controlled access zone.

The maximum containment laboratory has special engineering and containment features that will allow safe conduct of activities involving biohazardous agents that are extremely hazardous to laboratory workers or that may cause serious epidemic disease. Although the maximum security lab is usually a separate building, it can be constructed as an isolated area within a building. The distinguishing characteristic is the provision for secondary barriers to prevent hazardous materials from escaping into the environment. Such barriers include: sealing all lab openings, installing air locks or liquid disinfectant barriers, adding a contiguous clothing changing room, double door autoclave, biological waste treatment system, separate ventilation system and an exhaust air decontamination system.

## **IV General Biosafety Practices and Procedures**

### **a. Administrative controls**

#### **i Medical surveillance and examinations**

Departments must provide all employees who work with biohazardous agents an opportunity to receive medical attention, including any follow-up examinations deemed necessary by the examining physician, which will:

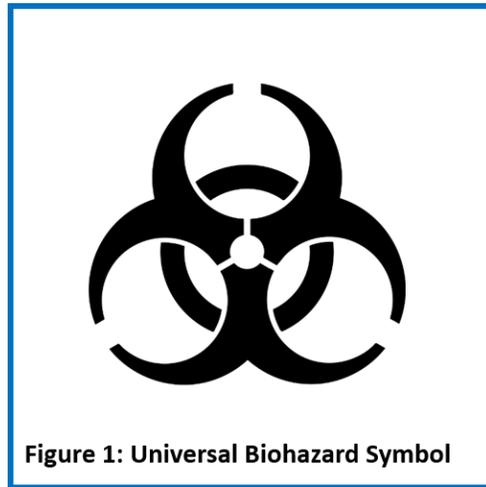
- Provide specific immunizations necessary to protect employee health.
- Evaluate the effects of possible exposure to a hazardous agent (e.g., symptoms of clinical infection, allergic symptoms or complaints related to pharmacological effects of end products, by-products, medium components or inactivated biological agents).

- Detect changes in employee health that may indicate the need for a change in job procedures or job assignment.
- Detect patterns of disease in the work force or evaluate the effectiveness of control measures.

University Employee Health Clinic (UEHC) located on the first floor of Boyd Tower in University of Iowa Hospitals and Clinics (UIHC), provides these services. They can be reached at 356-3631 (Monday-Friday 8:00-4:00pm).

## ii Signage / labeling

All areas and laboratories that contain biohazardous agents must be posted with a sign containing the universal biohazard symbol (Figure 1). Laboratory supervisors and PIs must ensure that labels on incoming containers of biohazardous agents are not removed or defaced. Laboratory containers, including bottles, flasks, sample vials, etc., must be marked, labeled or coded in all cases. The label should be dated and should identify the owner of the agent.



## b. General laboratory safety procedures

A few general laboratory practices can significantly decrease the likelihood of accidents occurring in the laboratory. Procedures to follow when working in any laboratory include:

- Be familiar with the materials you are working with (e.g., chemical, biological, radioactive). Refer to written laboratory protocols and review the SDS (Safety Data Sheets) for chemicals. Consider the toxicity of the materials and the health and safety hazards of each procedure (e.g., generation of aerosols). Take advantage of the knowledge and experience of laboratory personnel and the safety equipment that is available.
- Know the location of safety equipment and emergency procedures in your area.

- Always wear appropriate clothing (e.g., pants, shirts, shoes) in the laboratory. Open sandals are prohibited; shorts and skirts are not recommended.
- Do not work alone in the laboratory. When hazardous operations are conducted, arrangements should be made to have another person present in the lab.
- Keep the laboratory and work area clean and uncluttered.
- Work with all hazardous chemicals inside a fume hood.
- Never eat, smoke, drink, chew gum, prepare food or apply cosmetics in the laboratory.
- Do not leave reactions unattended.
- Prohibit unauthorized individuals from entering the laboratory.

### **c. Good microbiological techniques**

In addition to preventing general accidents, laboratory workers must also be aware of the characteristics and potential hazards of the microorganisms in the laboratory. Good microbiological techniques (GMT) can describe a range of techniques and procedures that when applied in a laboratory setting will prevent the contamination of the laboratory workers and the environment, as well as the experiment. GMT includes:

- Using proper aseptic techniques.
- Wearing appropriate personal protective equipment.
- Minimizing the production of aerosols or working in a BSC when aerosol production is unavoidable.
- Employing universal precautions with bloodborne pathogens.
- Routinely disinfecting work surfaces and equipment/supplies used in the experiment.
- Immediately cleaning spills according to laboratory protocols.
- Restricting work with infectious material to one area of the laboratory.
- Properly disposing of waste.
- Routinely washing hands following any experiment and after removing gloves.
- Reporting all accidents/incidents and seeking medical attention if necessary.

Further details regarding these techniques and protocols will be discussed throughout this manual. GMT should be applied when working with any microorganism, regardless of the agent's risk group.

### **d. Physical / engineering controls**

Safety equipment (primary barriers) includes BSCs, enclosed containers and other engineering controls designed to remove or minimize exposures to hazardous biological agents. Users of biohazardous agents must ensure BSCs and other protective equipment are adjusted and functioning properly prior to initiating an activity requiring their use. The design and construction of the facility (secondary barriers) also contributes to the laboratory workers' protection in addition to providing protection to

persons outside of the laboratory and within the community from biohazardous agents, which may be accidentally released from the laboratory.

## **i Biological safety cabinets**

BSCs are among the most effective and commonly used primary containment devices in laboratories working with biohazardous agents. There are three classes of BSCs, each having different performance characteristics as outlined below.

The Class I BSC provides protection to personnel and to the environment; however, product protection is not provided. The Class I BSC draws unfiltered room air into the work opening and across the work surface. This constant movement of air into the cabinet provides protection to the user (personnel), but because the air is unfiltered, there is no protection to the experimental materials (product). The air from inside the cabinet passes through a high efficiency particulate air (HEPA) filter prior to being exhausted, thus providing protection to the laboratory area (environment). Class I BSCs are no longer being manufactured on a regular basis and the vast majority have been replaced by Class II BSCs.

The Class II BSC provides product, personnel, and environmental protection. Like Class I BSCs, the Class II BSC utilizes constant inflow of air to provide personnel protection and HEPA-filtration of exhaust air to provide environmental protection. However, the Class II BSC also utilizes HEPA-filtered recirculating airflow within the work space to provide product protection.

The Class III BSC, commonly referred to as a glove box, is a self-contained gas-tight enclosure that provides a complete barrier between the worker and the materials in the cabinet. It is designed for use with high risk biologicals. When in use, it is maintained under negative air pressure. Supply and exhaust air are HEPA-filtered. Exhaust air is discharged outside the facility by dedicated exhaust fans.

## **ii Proper use and operation of the BSC according to cabinet class**

Class II BSCs, when used in conjunction with GMT, provide an effective partial containment system for safe manipulation of moderate and high-risk microorganisms (e.g., risk group 2 and 3 agents). The use of these cabinets alone is not appropriate for containment of the highest risk biohazardous agents because aerosols may accidentally escape through the open front. Class II BSCs are subdivided into two types (A and B) based on construction, air flow velocities and patterns, and exhaust systems. Basically, Type A cabinets are suitable for work with microbiological research in the absence of volatile or toxic chemicals and radionuclides, since air is recirculated within the work area.

Type A cabinets are exhausted through HEPA filters into the laboratory or may be exhausted to the outside, if a canopy (a.k.a. thimble) connection to the exhaust ductwork is used. Type B cabinets are hard-ducted to the exhaust system and contain negative pressure plena. These features, plus an increased face velocity of 100 feet per minute, allow work to be done with toxic/volatile chemicals or radionuclides. Type B cabinets are further sub-typed into two types, B1 and B2. Type B1 requires 70% of the downward-flowing air stream to be exhausted to the outside and 30% recirculated. Type B2 has 100% of the downward-flowing air exhausted to the outside; no air is recirculated within the cabinet. Call EHS for information on a comparison of the design features and applications.

Class III BSCs are totally enclosed, ventilated cabinets of gas-tight construction and offer the highest degree of personnel, environmental and product protection. Class III cabinets are most suitable for work with hazardous agents that require Biosafety Level 3 or 4 containment. The Class III cabinet is operated under negative pressure. Supply air is HEPA-filtered and the cabinet exhaust air is filtered by two HEPA filters in series, or HEPA filtration followed by incineration, before discharge outside the facility. All operations in the cabinet are performed through attached rubber gloves. Any equipment required by the laboratory activity, such as incubators, refrigerators and centrifuges must be an integral part of the system. Double-ended autoclaves and chemical dunk tanks are also attached to the cabinet system to allow supplies and equipment to be safely introduced and removed.

As with any other piece of laboratory equipment, personnel must be trained in the proper use of BSCs. Of particular note are those activities that may disrupt inward directional airflow through the work opening and allow the escape of aerosolized particles from within the cabinet (e.g., repeated insertion and withdrawal of worker's arms into and from the work chamber, opening and closing laboratory doors, improper placement of materials, improper operation of equipment within the work chamber or brisk walking past the cabinet while it is in use). These cabinets should be located away from traffic patterns and doors. Fans, heating and air conditioning registers and other air handling devices can also disrupt airflow patterns if located adjacent to a BSC. Strict adherence to recommended practices for use of BSCs and proper placement in the laboratory are as important in attaining the maximum containment capability of equipment as is the mechanical performance.

**IMPORTANT:** Cabinets must be tested and certified at the time of installation, any time they are moved, following internal repair, and at least annually

thereafter. Cabinets must be decontaminated prior to moving and certain repairs/maintenance. Certification is site specific and is required before the cabinet is put into operation in the laboratory. Please call the Biosafety Specialist at EHS (353-7702) with any questions regarding decontamination or certification of BSCs, or to schedule repair, certification, or decontamination.

### iii **Laminar flow hoods (a.k.a. “clean benches”)**

Laminar flow hoods (a.k.a. “clean benches”) utilize HEPA-filtered supply air to provide product protection. It is important that users are aware of the differences between clean benches and BSCs. Clean benches DO NOT provide personnel or environmental protection and therefore must NEVER be used with hazardous agents.

#### *Horizontal flow clean benches*

These clean benches discharge HEPA-filtered air from the back of the hood, across the work surface, and toward the user. They are present in a number of clinical, pharmaceutical and laboratory facilities where non-hazardous product protection is needed. They provide a high quality environment within the work chamber for manipulation of non-hazardous materials.

#### *Vertical flow clean benches*

These clean benches discharge HEPA-filtered air from the top of the hood, down to and across the work surface. Like their horizontal flow counterparts, these clean benches are useful in environments that require clean areas for protection of non-hazardous products (i.e. preparation of intravenous solutions). While these hoods have a sash and look similar to a BSC, they are not a substitute for a BSC and must never be used with hazardous materials.

### iv **Working safely in a biological safety cabinet**

#### *General*

- General precautions and guidelines used outside containment equipment apply to work performed in a BSC.
- BSCs should not be used in place of a chemical fume hood. Toxic and volatile chemicals are prohibited from Class II Type A BSCs. These chemicals as well as biological toxins should be manipulated in a chemical fume hood, or in a Class II Type B2 BSC if biohazardous agents are also present.
- Plan ahead to ensure minimal disruptions including room traffic or entry/exit, and restocking of supplies or materials in the cabinet.
- Open flames may not be used.
- Personnel should be trained by laboratory staff in proper BSC use.

### *Personal protection*

- Wear protective gloves when handling biohazardous agents. Change gloves if they become contaminated. A “double-glove” technique is advisable when working in a BSC, allowing users to change contaminated gloves without interrupting cabinet airflow.
- PPE is still necessary when using a BSC. Gloves and a lab coat are the minimal PPE required when working in a BSC. The necessary level of PPE should be determined by a risk assessment of the work being done.

### *Material Placement*

- The front intake grill of the Class II BSC must not be blocked with paper, equipment or other items.
- All materials, including aerosol-generating equipment, should be placed towards the rear of the cabinet without blocking the rear grill.
- Active work should flow from clean to contaminated areas across the work surface.
- An autoclavable biohazard bag and pipette collection tray should be placed inside the cabinet as frequent in-and-out movements when using these containers disrupts the integrity of the cabinet’s air barrier and can compromise both personnel and product protection.

### *Work practices*

- Move arms and hands slowly in the cabinet, wait one minute for air currents to settle, then begin work.
- Don't place anything on the grillwork inside the cabinet. This greatly interferes with the airflow.
- Decontaminate work surfaces with an appropriate disinfectant before and after work, and after a spill.
- Perform all procedures carefully to avoid the generation of aerosols, splashes and spills.
- Keep an appropriate disinfectant agent within the cabinet.
- Keep the number of items in a BSC to an absolute minimum.

### *Decontamination and Spills*

See [Section VIII](#)

### *Ultraviolet (UV) Lights*

American Biological Safety Association (ABSA), CDC, NIH and National Sanitation Foundation (NSF) agree that UV lights are not recommended in BSCs. Several factors influence the effectiveness of UV lights, including:

- Humidity – above 70 percent germicidal activity is drastically reduced.
- Dynamic air flow within the cabinet – reduces the penetration of the light and also cools the lamps, resulting in reduced output.
- Age – effective UV radiation emitted from the lamps decreases with age.

If used:

- The light must only be used when the BSC sash is fully closed. Sash alarms or mechanisms ensuring this feature may not be disabled.
- All items to be removed from the BSC and surfaces of the BSC must be decontaminated prior to UV light use.
- They must be cleaned weekly (alcohol/water mixture) in order to remove dust and dirt that can block the germicidal effectiveness of the light.
- UV light intensity should be checked or the light replaced annually to ensure the emitted intensity is sufficient for germicidal activity.
- UV lights must be turned off whenever the room is occupied.
- If UV lights will be used overnight or for extended periods of time while staff are not present, signage should be posted on the closed door of the room.

### *Cabinets*

Cabinet selection should be determined by assessing personnel protection against risk group 1-4 agents, personnel protection against radionuclides and volatile toxic chemicals, product protection, and the environment; or a combination of these. HEPA filters do not provide protection against gases and vapors; they do provide protection against particulate agents and materials. BSCs must be certified after installation, but before being used, whenever moved, after certain repairs and at least annually thereafter.

## **v Laboratory vacuum lines**

Proper set-up and maintenance of laboratory vacuum lines are important to protect the central vacuum system as well as facility maintenance staff who service this equipment. The vacuum system should be protected by a HEPA filter, particularly when used to manipulate biohazardous materials, and an overflow container should be present and managed vigilantly. Lab materials should never reach the main vacuum pump and potentially expose maintenance staff to biohazardous/chemical agents.

For activities conducted in biosafety cabinets/tissue culture hoods the recommended aspiration set-up is illustrated below in Figure 2. The set-up includes a collection or suction vessel (A), an overflow collection vessel (B), and a vacuum protection in-line hydrophobic HEPA filter (C) placed immediately before the valve (D). Sufficient chemical disinfectant is placed in the primary collection and overflow vessels to inactivate biological agents as they are collected. Spargers (or tubes, as shown in B) in the vessels are also recommended to minimize aerosols being directly sucked into the vacuum line without being disinfected. An example of a commonly selected HEPA filter is the Whatman® VACU-GUARD. Primary collection flasks should be disposed of daily, or more often as necessary; HEPA filters should be placed in a periodic

inspection and replacement schedule. Collections flasks should be labeled with the universal biohazard symbol; if collection flasks are stored on the floor, secondary containment should be used.

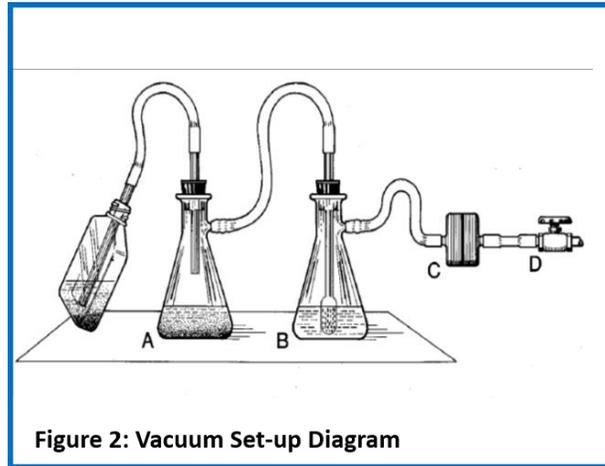


Figure 2: Vacuum Set-up Diagram

### e. Personal protective equipment (PPE)

The purpose of PPE is to act as a barrier to minimize the risk of exposure to aerosols, splashes and accidental inoculation. Exposure may occur via apparent or unapparent skin lesions or through the membranes of the eye, nose or mouth. PPE is often used in combination with BSCs and other equipment that contain the agents or animals being handled. PPE should be worn only while working in the laboratory; all PPE should be removed and hands should be washed before exiting the laboratory. Examples of PPE and the protection afforded are listed below in Table 2.

Table 2. Personal Protective Equipment

PPE	Hazard Corrected	Safety Features
Laboratory coats, gowns, coveralls	Contamination of clothing	Cover street clothing
Plastic aprons	Contamination of clothing	Fluid-proof
Goggles or safety glasses	Impact and splash (eyes)	Impact-resistant lenses (must be optically correct or worn over eye glasses) Side shields
Face shields	Impact and splash (face)	Shields entire face (i.e. mucus membrane protection) Easily removable
Respirators	Inhalation of aerosols	Designs include hood, full face, or half-face masks.
Gloves	Accidental direct contact	Hand protection

PPE	Hazard Corrected	Safety Features
	Accidental punctures or cuts (depending on glove material) Chemical or thermal burns (depending on glove material)	Several disposable options (latex, vinyl, or nitrile) Puncture resistant (depending on material) Chemical resistant (depending on material) Thermal resistant (depending on material)

## i Selection precautions

- Equipment should be selected based on the specific work, exposure conditions that will be encountered and the anticipated level of risk. Evaluate the task, the exposure associated with its performance and select appropriate PPE that will be an effective barrier under anticipated conditions of exposure.
- PPE must be made available in the proper size.
- Laundering and repair or replacement must be available for those items requiring it.
- Individuals must not launder items at home.
- Proper disposal containers for contaminated equipment must be in place.
- The limitations of the equipment must be understood.

## ii Protective clothing

Lab coats, gowns, or fluid-proof aprons, should be worn in laboratory settings. We strongly recommend lab coats and gowns with fitted cuffs (knitted or elastic) to provide maximal arm protection. Long sleeved garments are preferred to minimize contamination of skin or street clothes and to reduce shedding of microorganisms from the arms. Back-opening gowns offer better protection than lab coats and are required when working in a BSL3 laboratory. Aprons should be worn over lab coats to provide additional protection from spills and chemicals. Additional criteria for selecting clothing are: comfort, appearance, closures, anti-static properties and durability. Consideration must be given to having disposable clothing available for visitors, maintenance and service workers.

Reusable protective clothing such as lab coats should be laundered on a regular basis to maintain hygiene. Laundering should be completed whenever the lab coat is visibly soiled. The frequency and manner in which lab coats are used, determine how often they should be laundered. Laundering of reusable protective clothing shall be performed by [The University of Iowa Laundry Service](#); laundering **may not** be done by lab staff members at private residences or public laundry facilities.

Any lab coat/protective clothing known or reasonably suspected to be contaminated with biohazardous material(s) must be decontaminated (i.e. autoclaved or treated with an effective disinfectant) before it is sent to laundry services. If protective clothing will be autoclaved, it must be capable of withstanding high temperatures. **Do not autoclave lab coats that are additionally contaminated with chemical or radioactive materials;** dispose of the contaminated clothing as hazardous waste. Additionally, lab coats that become grossly contaminated with biohazardous material(s) should be disposed of as biohazardous waste.

### iii **Hand protection**

Gloves must be worn when working with infectious agents, blood, body fluids and chemicals. Gloves must be selected based on the hazards involved and the activity to be conducted. Temperature resistant gloves must be worn when handling hot material or dry ice. Delicate work requiring a high degree of precision dictates the use of thin-walled gloves. Table 3 below lists some general characteristics for commonly used gloves. You may contact EHS for assistance in selecting the appropriate gloves for protection from toxic or corrosive chemicals.

The lower sleeve or cuff of the laboratory garment should be overlapped by the glove. A long cuffed glove or disposable arm shield may be worn for further protection of the garment or the work. When gloves become contaminated while working in a BSC they must be discarded into a waste container within the cabinet. Hands must be washed and new gloves donned before continuing the work.

Disposable gloves are not to be washed or disinfected for re-use. Disinfecting agents may cause deterioration of the glove material or compromise the protective qualities. They must be changed as soon as possible when visibly soiled. Replace gloves if a tear, puncture or similar defect is noticed. Place used gloves in a plastic bag or container marked with a biohazard symbol and bearing the word BIOHAZARD.

Some people develop an allergy to the latex and/or powder in gloves they are using. A variety of options are available that can alleviate allergic reactions. Both powdered and powder-free hypo-allergenic latex gloves are available from suppliers. Nitrile gloves are a good alternative for those with latex allergies.

Utility gloves (rubber gloves), often used for housekeeping chores, are of a more substantial construction than surgical or examination gloves and are permitted to be decontaminated and reused. They must be discarded if they are cracked, peeling, discolored, torn, punctured or exhibit other signs of deterioration.

**Table 3. General Characteristics for Commonly used Gloves**

Glove Material	Chemical/Biohazard
Butyl rubber	Nitric acid, sulfuric acid, hydrofluoric acid, red fuming nitric acid and peroxide Gases, most chemicals and water vapor
Latex (natural rubber)	Dilute water solutions of acids, alkalis, salts and ketones Blood, body fluids, infectious agents
Neoprene	Oils, greases, alcohols, resins, alkalis, organic acids and many solvents Corrosive chemicals
Nitrile (nitrile-butadiene)	Aromatic petroleum and chlorinated solvents Blood, body fluids, infectious agents Good alternative for those with latex allergies
Silver Shield or 4H gloves	HazMat work Glove of choice for a universal spill kit

**iv Eye and face protection**

Masks and eye protection or chin-length face shields must be worn whenever splashes, spray, spatter, droplets of blood or other potentially infectious materials (OPIM; as defined in the [Bloodborne Pathogens Standard](#), below) may be generated with a potential for mucous membrane contamination. Goggles should be worn over normal prescription eye glasses and contact lenses which do not provide protection against biological hazards. If eyewear is chosen over the use of a face shield, it must be worn in conjunction with a face mask, since the goal is to provide protection of the eyes, nose and mouth.

**v Respirators**

Where the use of a respirator is necessary to limit exposure to biohazardous agents, the department must provide, at no cost to the employee, the proper respiratory protective equipment. Only HEPA filters will provide protection against microorganisms and it is imperative that any filter be fitted in the correct type of respirator. Respirators must be selected and used in accordance with the requirements of the University's Respiratory Protection Program. Individuals required to wear a respirator will receive specific training and a fit test before being issued a respirator. In addition, individuals must undergo periodic medical monitoring. Contact EHS for further information.

**f. Work practice controls**

Work practice controls are meant to reduce the likelihood of exposure by altering the manner in which a task is performed. In other words, these controls are dependent upon employee behavior. It is necessary to employ good work practices in tandem with

effective engineering controls and personal protective equipment in order to achieve the goals of this program.

**i Standard operating procedure (SOP)**

This manual serves as a generic SOP relevant to safety and health concerns with work involving biohazardous agents. Area-specific operations should be included by the department, PI or supervisor. The SOP is intended to provide employees with the necessary guidelines for conducting work in a safe and consistent manner.

The types and quantity (as in large scale quantities) of organisms and the operations performed all define at which biosafety level the lab must function. Each level has certain recommended criteria in the areas of standard microbiological practices, special practices, safety equipment and laboratory facilities. See [Section VI](#) for biosafety level criteria and [Section VII](#) for the vertebrate animal biosafety level criteria.

Standard operating procedures should include the following provisions:

- Use of containment devices such as BSCs
- PPE requirements
- Procedures for proper disposal of contaminated waste
- Decontamination procedures
- Spill procedures

**ii Decontamination and disposal**

Please see [Section VIII](#) for detailed information on disinfection, sterilization and disposal.

**g. Bloodborne Pathogens (BBP) Standard**

All occupational exposure to blood or other potentially infectious materials (OPIM) is regulated under the Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Standard, Title 29 of the Code of Federal Regulations (29 CFR 1910.1030). Occupational exposure means reasonably anticipated skin, eye, mucous membrane or parenteral contact with blood or OPIM that may result from the performance of an employee's duties. The standard mandates the use of Universal Precautions as an approach to exposure control. According to this concept, all human blood and certain human body fluids are treated as if they were infected with human pathogens (such as HIV, HBV HCV, etc.). The universal precautions concept is only one part of the overall plan to reduce exposures. Other methods of control include engineering controls that isolate or remove the bloodborne pathogens hazard from the workplace (e.g., self-sheathing needles or needleless systems), work practice controls that alter the manner in which a task is performed, use of PPE, receipt of the hepatitis B vaccine and training in exposure control.

Potentially infectious materials as defined in the standard are:

- Human blood, blood components and blood products
- The following human body fluids: semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva in dental procedures, any body fluid that is visibly contaminated with blood, and all body fluids where it is difficult or impossible to differentiate between body fluids
- Any unfixed tissues or organs (other than intact skin) from a human (living or dead);
- HIV- or HBV-containing cell, organ, tissue cultures, culture mediums or other solutions
- Blood, organs or other tissue from animals infected with HIV or HBV.

It is the responsibility of the employer to make the hepatitis B vaccine available, at no cost, to all employees who have potential for occupational exposure. All post-exposure evaluations and follow-up will also be provided to employees at no cost. Employers must have documentation that all at-risk employees have been offered the hepatitis B vaccine. All "At Risk" employees should initially complete an online hepatitis B vaccination survey offered through UEHC's patient software, ReadySet, regardless of whether the employee is accepting or declining the vaccination. Instructions for creating a ReadySet account and completing the Hepatitis B vaccination survey is available on the EHS website, under "Forms". Staff accepting the vaccination should contact UEHC (356-3631) to schedule an appointment after completing the survey.

### **Bloodborne pathogens exposure control plan**

Every department must have a Bloodborne Pathogens Exposure Control Plan (ECP) which contains information specific to that department. An [ECP template](#) is available on EHS's web site. Information relevant to your particular situation or area will need to be added, with those sections indicated in the template by blue script. Each department's ECP must be reviewed on an annual basis and updated when necessary to reflect new or modified tasks and procedures that affect occupational exposure and to reflect new or revised employee positions with occupational exposure. An annual reminder to update the ECP will be sent to the Departmental Exposure Control Officers from the Biological Safety Office at EHS. Attached to this reminder will be an updated template and instructions on the changes to be made.

The full text of the [Bloodborne Pathogens Standard](#) can be found on OSHA's Web site.

## V Biosafety Level Criteria

Four biosafety levels (BSLs) have been defined that consist of specified lab practices and techniques, safety equipment and lab facilities. These are commensurate with the operations performed and with the hazard potential posed by the biohazardous agents with which the laboratory works.

The reference for this section is the Biosafety in Microbiological and Biomedical Laboratories ([BMBL](#)) 5th Edition, 2009, U.S. Department of Health and Human Services. Described below are BSLs 1-3. The maximum containment lab (BSL4) described in the BMBL is not included in this manual, as facilities do not presently exist at the University that would accommodate the safety regulations involved with Level 4 work. Table 4 presents a summary of the different biosafety level requirements.

**Table 4. Summary of biosafety level requirements**

Requirement	BSL1	BSL2	BSL3
Isolation of laboratory	No	No	No
Room sealable for decontamination	No	No	Yes
Ventilation			
Inward air flow	No	Desirable	Yes
Mechanical via building system	No	Desirable	Yes
Mechanical, independent	No	Desirable	Yes
HEPA-filtered exhaust air	No	No	Desirable
Double-door entry	No	No	Yes
Anteroom	No	No	Yes
Effluent treatment	No	No	No
Autoclave			
On site	No	Yes	Yes
In laboratory room	No	No	Desirable
Double-ended "pass-through"	No	No	Desirable
BSC (Class II)	No	Desirable	Yes

### a. BSL1: The basic laboratory

BSL1 is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to lab personnel or the environment. The laboratory is not necessarily separated from general building traffic patterns and work is usually conducted on open benchtops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by an appropriate risk assessment. Lab personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science.

## **i Standard microbiological practices**

- The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
- Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
- Eating, drinking, smoking, handling contact lenses, and applying cosmetics and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
- Mouth pipetting is prohibited; mechanical pipetting devices must be used.
- Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.
- Precautions, including those listed below, must always be taken with sharp items. These include:
  - Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
  - Used disposable needles and syringes 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, preferable by autoclaving.
  - Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.
- Perform all procedures to minimize the creation of splashes and/or aerosols.
- Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
- Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport.
  - Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.

- Materials to be removed from the facility for decontamination must be packaged in accordance with applicable local, state, and federal regulations.
- A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. The sign may include the name of the agent(s) in use and the name and phone number of the laboratory supervisor or other responsible personnel. Agent information should be posted in accordance with the institutional policy.
- An effective integrated pest management program is required. Please see Appendix G in the BMBL, 5th Edition.
- The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.
- EHS strongly discourages the use of cell phones and other personal electronic devices in the laboratory. When used in laboratories where hazardous materials are used, mobile devices can be both distracting and can become potential fomites for infectious agents and/or harmful chemicals. If you need to use a cell phone/personal electronic device, please ensure you remove PPE and wash your hands before handling the device.

**ii Special practices**

None

**iii Safety equipment (primary barriers)**

- Special containment devices or equipment, such as biological safety cabinets, are not generally required.
- Protective laboratory coats, gowns or uniforms are recommended to prevent contamination of personal clothing.
- Wear protective eyewear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses in laboratories should also wear eye protection.
- Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk

assessment. Alternatives to latex gloves should be available. Wash hands prior to leaving the laboratory. In addition, BSL-1 workers should:

- Change gloves when contaminated, integrity has been compromised, or when otherwise necessary.
- Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
- Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

#### **iv Laboratory facilities**

- Laboratories should have doors for access control.
- Laboratories must have a sink for hand washing.
- The laboratory should be designed so that it is easily cleaned. Carpets and rugs in laboratories are not appropriate.
- Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets and equipment should be accessible for cleaning.
- Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
- Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
- Laboratory windows that open to the exterior should be fitted with fly screens.

### **b. BSL2: The containment laboratory**

BSL2 builds upon BSL1 and is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL1 in that:

- Laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures.
- Access to the laboratory is restricted when work is being conducted.
- All procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

#### **i Standard microbiological practices (in addition to those for BSL1)**

- A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include: the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent

information should be posted in accordance with the institutional policy.

- The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures.
- Personnel must receive annual updates or additional training when procedural or policy changes occur.
- Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection.
- Individuals having these conditions should be encouraged to self-identify to the UEHC for appropriate counseling and guidance.

## **ii Special practices**

- All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
- Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.
- Each institution must establish policies and procedures describing the collection and storage of serum samples from at-risk personnel.
- A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
- The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.
- Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
- Laboratory equipment should be routinely decontaminated, as well as after spills, splashes, or other potential contamination.
- Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
- Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
- Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety safety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
- Animals and plants not associated with the work being performed must not be permitted in the laboratory.

- All procedures involving the manipulation of infectious materials that may generate an aerosol must be conducted within a BSC or other physical containment devices.

### iii **Safety equipment (in addition to those listed for BSL1)**

- Properly maintained biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices must be used whenever:
  - Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally and harvesting infected tissues from animals or eggs.
  - High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.
- Protective laboratory coats, gowns, smocks or uniforms designated for laboratory use must be worn while working with hazardous materials. Remove protective clothing before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). Dispose of protective clothing in the laboratory or deposit it for laundering by the University; protective clothing is not to be taken home by personnel.
- Eye and face protection (goggles, mask, face shield or other splatter guard) must be used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be manipulated outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.
- Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should:
  - Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
  - Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
  - Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
- Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment

**iv Laboratory facilities (in addition to the requirements for BSL1)**

- Laboratory doors should be self-closing and have locks in accordance with the institutional policies.
- Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.
- The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.
- Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.
- BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
- Vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
- An eyewash station must be readily available.
- There are no specific requirements on ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.
- HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a canopy (a.k.a. thimble) connection (Type A cabinets) or a direct (a.k.a. hard) connection (Type B cabinets). Provisions to assure proper safety cabinet performance and air system operation must be verified.
- A method for decontaminating all laboratory wastes should be available in the facility (e.g. autoclave, chemical disinfection, incineration, or other validated decontamination method).

**c. BSL3: The high containment laboratory**

BSL3 is applicable to clinical, diagnostic, teaching, research or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through inhalation route exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents and must be supervised by scientists competent in handling infectious agents and associated procedures. All procedures involving the manipulation of infectious material must be conducted within a BSC or other physical containment device or by personnel wearing

appropriate personal protective equipment. A BSL3 laboratory has special engineering and design features.

**i Standard microbiological practices (in addition to those for BSL1 & BSL2)**

- Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method). Depending on where the decontamination will be performed, the following methods should be used prior to transport:
  - Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
  - Materials to be removed from the facility for decontamination must be packaged in accordance with applicable local, state, and federal regulations.

**ii Special practices (in addition to those listed for BSL2)**

- The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL3 agents.
- All procedures involving the manipulation of infectious materials must be conducted within a BSC or other physical containment devices. No work with open vessels is conducted on the bench. When a procedure cannot be performed within a BSC, a combination of personal protective equipment and other containment devices, such as a centrifuge safety cup or sealed rotor, must be used.

**iii Safety equipment**

- All procedures involving the manipulation of infectious materials must be conducted within a BSC (preferably Class II or Class III), or other physical containment devices.
- Protective lab clothing with a solid-front such as tie-back or wrap-around gowns, scrub suits, or coveralls are worn by workers when in the laboratory. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated with appropriate disinfectant before being laundered. Clothing is changed when contaminated.
- Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories must also wear eye protection.

- Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-3 laboratory workers should:
  - Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
  - Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
  - Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
- Eye, face and respiratory protection must be used in rooms containing infected animals.

#### **iv Laboratory facilities**

- Laboratory doors must be self-closing and have locks in accordance with the institutional policies. The laboratory must be separated from areas that are open to unrestricted traffic flow within the building. Access to the laboratory is restricted to entry by a series of two self-closing doors. A clothing change room (anteroom) may be included in the passageway between the two self-closing doors.
- Laboratories must have a sink for hand washing. The sink must be hands-free or automatically operated. It should be located near the exit door. If the laboratory is segregated into different laboratories, a sink must also be available for hand washing in each zone. Additional sinks may be required as determined by the risk assessment.
- The laboratory must be designed so that it can be easily cleaned and decontaminated. Carpets and rugs are not permitted. Seams, Floors, walls, and ceiling surfaces should be sealed. Spaces around doors and ventilation openings should be capable of being sealed to facilitate space decontamination.
  - Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Consideration should be given to the installation of seamless, sealed, resilient or poured floors, with integral cove bases.
  - Walls should be constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.
  - Ceilings should be constructed, sealed, and finished in the same general manner as walls.
- Decontamination of the entire laboratory should be considered when there has been gross contamination of the space, significant changes in laboratory usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to

decontaminate the laboratory must be based on the risk assessment of the biological agents in use.

- Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets and equipment should be accessible for cleaning.
  - Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
  - Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
- All windows in the laboratory must be sealed.
- BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
- Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
- An eyewash station must be readily available in the laboratory.
- A ducted air ventilation system is required. The system must provide sustained directional airflow by drawing air in to the laboratory from “clean” areas toward “potentially contaminated” areas. The laboratory shall be designed such that under failure conditions the airflow will not be reversed.
  - Laboratory personnel must be able to verify directional airflow. A visual monitoring device which confirms directional air flow must be provided at the laboratory entry. Audible alarms should be considered to notify personnel of air flow disruption.
  - The laboratory exhaust air must not re-circulate to any other area of the building.
  - The laboratory building exhaust air exhaust should be dispersed away from occupied areas and from building air intake locations or the exhaust air must be HEPA filtered.
- HEPA-filtered exhaust air from Class II biological safety cabinet can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer’s recommendations. Biological safety cabinets can also be connected to the laboratory exhaust system by either a canopy (a.k.a. thimble) connection (Type A cabinets) or a direct (a.k.a. hard) connection (Type B cabinets). Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be certified at least annually to assure correct performance. Class III BSCs must be directly (hard) connected up through the second exhaust HEPA filter of the cabinet. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.

- A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration or other validated decontamination method).
- Equipment that may produce infectious aerosols must be contained in devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters should be tested and/or replaced at least annually.
- Facility design consideration should be given to means of decontaminating large pieces of equipment before removal from the laboratory.
- Enhanced environmental and personal protection may be required by the agent summary statement, risk assessment, or applicable local, state, or federal regulations. These laboratory enhancements may include, for example, one or more of the following; an anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities; gas tight dampers to facilitate laboratory isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; and advanced access control devices such as biometrics. HEPA filter housings should have gas-tight isolation dampers; decontamination ports; and/or bag-in/bag-out (with appropriate decontamination procedures) capability. The HEPA filter housing should allow for leak testing of each filter and assembly. The filters and the housing should be certified at least annually.
- The BSL3 facility design, operational parameters, and procedures must be verified and documented prior to operation. Facilities must be re-verified and documented at least annually.

**d. Biosafety level and risk group relationships**

Table 5 presents an overview of the relationship between risk groups, biosafety levels, practices and equipment.

**Table 5. Relation of risk groups to biosafety levels, practices and equipment**

<b>Risk Group</b>	<b>Biosafety Level</b>	<b>Laboratory type</b>	<b>Laboratory practices</b>	<b>Safety equipment</b>
<b>1</b>	BSL1: Basic	Basic teaching, research	GMT <sup>1</sup>	None; open bench work
<b>2</b>	BSL2: Containment	Primary health services; diagnostic, research	GMT <sup>1</sup> plus protective clothing, biohazard signage	Open bench plus BSC for potential aerosols
<b>3</b>	BSL3: High containment	Special diagnostic, research	All level 2 practices, plus special clothing, controlled	BSC and/or other primary containment

Risk Group	Biosafety Level	Laboratory type	Laboratory practices	Safety equipment
			access, directional airflow	devises for all activities
4	BSL4: Maximum containment	Dangerous pathogen units	All level 3 practices, plus airlock entry, shower exit, special waste disposal	Class III BSC or positive pressure suites in conjunction with Class II BSCs, double-ended “pass-through” autoclave, HEPA-filtered air

<sup>1</sup>GMT: Good Microbiological Techniques (Section V)

## VI Animal Biosafety Criteria

These guidelines describe three combinations of practices, safety equipment and facilities for experiments on animals infected with agents that produce or may produce human infection. They provide increasing levels of protection to personnel and the environment and are recommended as minimal standards for activities involving infected laboratory mammals. These three combinations are designated in each of the Animal Biosafety Levels (ABSL) 1 through 3 and describe animal facilities and practices applicable to work on animals infected with agents assigned to corresponding BSL1 through 3.

The reference for this section is the [BMBL](#), 5th Edition, 2009, U.S. Department of Health and Human Services. See Appendix Q of the [NIH Guidelines](#) for additional requirements specific to large animals involved in research with rDNA.

### a. Vertebrate ABSL1

ABSL1 is used for research with well characterized agents that are not known to cause disease in immunocompetent adult humans and present minimal potential hazard to laboratory personnel and the environment. ABSL-1 facilities should be separated from the general traffic patterns of the building and restricted as appropriate. Special containment equipment or facility design may be required as determined by appropriate risk assessment. Personnel must have specific training in animal facility procedures and must be supervised by an individual with adequate knowledge of potential hazards and experimental animal procedures.

## **i Standard microbiological practices**

- The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergency situations. Worker safety and health concerns are addressed as part of the animal protocol review. Each project is subject to approval by the IACUC (Institutional Animal Care and Use Committee), and possibly the Institutional Biosafety Committee (IBC).
- A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety professionals. The safety manual must be available and accessible. Personnel are advised of potential hazards and are required to read and follow instructions on practices and procedures.
- Supervisors must ensure that animal care, laboratory and support personnel receive appropriate training regarding their duties, animal husbandry procedure, potential hazards, manipulations of infectious agents, necessary precautions to prevent hazard or exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates or additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.
- Appropriate medical surveillance program is in place, as determined by risk assessment. The need for an animal allergy prevention program should be considered. Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care and manipulations.
- Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.
- Personnel using respirators must be enrolled in an appropriately constituted respiratory protection program.
- A sign incorporating safety information must be posted at the entrance to the areas where infectious materials and/or animals are housed or are manipulated. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of specific infectious agents is recommended when more than one agent is being used within an animal room.

- Security-sensitive agent information should be posted in accordance with the institutional policy. Advance consideration should be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.
- Access to the animal room is limited. Only those persons required for program or support purposes are authorized to enter the facility. All persons including facility personnel, service workers, and visitors are advised of the potential hazards (natural or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.
- Protective laboratory coats, gowns or uniforms are required to prevent contamination of personal clothing. Gloves are worn to prevent skin contact with contaminated, infectious and hazardous materials, and when handling animals. Gloves and personal protective equipment should be removed in a manner that minimizes transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated. Persons must wash their hands after removing gloves and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Eye and face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics and storing food for human use should only be done in designated areas and are not permitted in animal or procedure rooms.
- All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
- Mouth pipetting is prohibited. Mechanical pipetting devices must be used.
- Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:
  - Needles and syringes or other sharp instruments are limited to use in the animal facility when there is no alternative for such procedures as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
  - Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the work site as possible.
  - Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferable by autoclaving.

- Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.
- Equipment containing sharp edges and corners should be avoided.
- Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent, and after any spills, splashes, or other overt contamination.
- Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/or animals are housed or are manipulated.
- An effective integrated pest management program is required. Please see Appendix G in the BMBL, 5th Edition.
- All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof, covered containers for appropriate disposal in compliance with applicable institutional, local and state requirements. Decontaminate all potentially infectious materials before disposal using an effective method.

## **ii Special practices**

None

## **iii Safety equipment (primary barriers)**

- A risk assessment should determine the appropriate type of personal protective equipment to be utilized. Protective laboratory coats, gowns or uniforms are recommended to prevent contamination of personal clothing. Protective outer clothing is not worn outside areas where infectious materials and/or animals are housed or manipulated. Gowns and uniforms are not worn outside the facility.
- Protective eyewear is worn when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates. Persons having contact with non-human primates should assess risk of mucus membrane exposure and wear appropriate protective equipment (e.g., masks, goggles, face shields, etc.) as needed.
- Gloves should be worn to protect hands from exposure to hazardous materials. A risk assessment should be performed to identify the appropriate glove for the task and alternatives to latex gloves should be available. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Gloves must not be worn outside of the animal rooms.
- Gloves and personal protective equipment should be removed in a manner that prohibits transfer of infectious materials. Do not wash or

reuse disposable gloves. Dispose of used gloves with other contaminated waste.

- Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Hand washing should occur after the removal of gloves.

#### **iv Animal facilities (secondary barriers)**

- The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking.
- Access to the animal facility is restricted. Doors to areas where infectious materials and/or animals are housed open inward, are self-closing, are kept closed when experimental animals are present, and should never be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.
- The animal facility must have a sink for hand washing. Sink traps are filled with water, and/or appropriate liquid to prevent the migration of vermin and gases.
- The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors, and ceilings) are water resistant. It is recommended that penetrations in floors, walls and ceiling surfaces are sealed, to included openings around ducts, doors and door frames, to facilitate pest control and proper cleaning. Floors must be slip resistant, impervious to liquids, and resistant to chemicals.
- Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets and equipment should be accessible for cleaning. Chairs used in the animal area must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Sharp edges and corners should be avoided.
- External windows are not recommended; if present windows must be resistant to breakage. Where possible, windows should be sealed. If the animal facility has windows that open, they should be fitted with fly screens. The presence of windows may impact facility security and therefore should be assessed by security personnel.
- Ventilation should be provided in accordance with the Guide for Care and Use of Laboratory Animals. No recirculation of exhaust air should occur. It is recommended that animal rooms have inward directional airflow. Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.
- Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.

- If floor drains are provided, the traps are always filled with water, and/or an appropriate disinfectant to prevent the migration of vermin and gases.
- Cages are washed manually or preferably in a mechanical cage washer. The mechanical cage washer should have a final rinse temperature of at least 180°F.
- Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
- Emergency eyewash and shower are readily available; location is determined by risk assessment.

## **b. Vertebrate ABL2**

ABSL2 is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment. Exposure via ingestion, percutaneous and mucous membranes is also addressed.

### **i Standard practices (in addition to those listed for ABSL1)**

- A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety professionals. The safety manual must be available and accessible. Personnel are advised of potential hazards and are required to read and follow instructions on practices and procedures. Consideration should be given to specific biohazards unique to the animal species and protocol in use.
- Access to the animal room is limited. Only those persons required for program or support purposes are authorized to enter the animal facility and the areas where infectious materials and/or animals are housed or are manipulated. All persons including facility personnel, service workers, and visitors are advised of the potential hazards (natural or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.

### **ii Special practices**

- Animal care staff, laboratory and routine support personnel must be provided a medical surveillance program as dictated by the risk assessment, and administered appropriate immunizations for agents handled or potentially present, before entry into animal rooms. When appropriate, a base line serum sample should be stored.
- Procedures involving a high potential for generating aerosols should be conducted within a biosafety cabinet or other physical containment device. When a procedure cannot be performed within a biosafety cabinet, a combination of personal protective equipment and other containment devices must be used. Consideration should be given to the use of restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint devices, chemical restraint medications, etc.).

- Decontamination is recommended for all potentially infectious materials and animal waste before movement outside the areas where infectious materials and/or animals are housed or are manipulated by an appropriate method (e.g. autoclave, chemical disinfection, or other approved decontamination methods). This includes potentially infectious animal tissues, carcasses contaminated bedding, unused feed, sharps, and other refuse. Consideration should be given to means for decontaminating routine husbandry equipment, sensitive electronic and medical equipment.
- Materials to be decontaminated outside of the immediate areas where infectious materials and/or animals are housed or are manipulated must be placed in a durable, leak proof, covered container and secured for transport. The outer surface of the container is disinfected prior to moving materials. The transport container must contain a universal biohazard label. Develop and implement an appropriate waste disposal program in compliance with applicable institutional, local and state requirements. Autoclaving of content prior to incineration is recommended.
- Equipment, cages, and racks should be handled in a manner that minimizes contamination of other areas. Equipment must be decontaminated before repair, maintenance, or removal from the areas where infectious materials and/or animals are housed or are manipulated. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
- Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the safety manual. All such incidents must be reported to the animal facility supervisor or personnel designated by the institution. Medical evaluation, surveillance, and treatment should be provided as appropriate and records maintained.

### **iii Safety equipment (in addition to those listed for ABSL1)**

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

removed before leaving the animal facility. Reusable clothing is appropriately contained and decontaminated before being laundered. Laboratory and protective clothing should never be taken home.

- Gowns, uniforms, laboratory coats, and personal protective equipment are worn while in the areas where infectious materials and/or animals are housed or manipulated and removed prior to exiting. Disposable personal protective equipment and other contaminated waste are appropriately contained and decontaminated prior to disposal.
- Eye and face protection (goggles, mask, face shield or other splatter guard) are used for anticipated splashes/sprays from infectious or other hazardous materials when the animal or microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates.

#### **iv Animal facilities (in addition to those listed for ABSL1)**

- A hand washing sink is located at the exit of the areas where infectious materials and/or animals are housed or are manipulated. Additional sinks for hand washing should be located in other appropriate locations within the facility. If the animal facility has segregated areas where infectious materials and/or animals are housed or manipulated, a sink must also be available for hand washing at the exit from each segregated area.
- Sink traps are filled with water, and/or appropriate liquid to prevent the migration of vermin and gases.
- The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors, and ceilings) are water resistant. It is recommended that penetrations in floors, walls and ceiling surfaces are sealed, to included openings around ducts, doors and door frames, to facilitate pest control and proper cleaning. Floors must be slip resistant, impervious to liquids, and resistant to chemicals.
- External windows are not recommended; if present, windows should be sealed and must be resistant to breakage. The presence of windows may impact facility security and therefore should be assessed by security personnel.
- Ventilation should be provided in accordance with the Guide for Care and Use of Laboratory Animals. The direction of airflow into the animal facility is inward; animal rooms should maintain inward directional airflow compared to adjoining hallways. A ducted exhaust air ventilation system is provided. Exhaust air is discharged to the outside without being recirculated to other rooms. Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.

- Cages should be autoclaved or otherwise decontaminated prior to washing. The mechanical cage washer should have a final rinse temperature of at least 180°F. The cage wash area should be designed to accommodate the use of high pressure spray systems, humidity, strong chemical disinfectants and 180°F water temperatures, during the cage/equipment cleaning process.
- If BSCs are present, they must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer’s recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. Correct performance of the BSCs should be recertified at least once a year.
- All BSCs should be used according to manufacturer’s recommendations, to protect the worker and avoid creating a hazardous environment from volatile chemicals and gases.
- If vacuum service (i.e., central or local) is provided, each service connection should be fitted with liquid disinfectant traps and an inline HEPA filter, placed as near as practicable to each use point or service cock. Filters are installed to permit in place decontamination and replacement.
- An autoclave should be considered in the animal facility to facilitate decontamination of infectious materials and waste.

**c. Vertebrate ABSL3**

ABSL3 involves practices suitable for work with laboratory animals infected with indigenous or exotic agents, agents that present a potential for aerosol transmission, and agents causing serious or potentially lethal disease. ABSL3 builds upon the standard practices, procedures, containment equipment, and facility requirements of ABSL2.

**i Standard practices (in addition to those listed for ABSL1 and 2)**

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

- Security-sensitive agent information and occupational health requirements should be posted in accordance with the institutional policy. Advance consideration should be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.
- Access to the animal room is limited to the fewest number of individuals possible. Only those persons required for program or support purposes are authorized to enter the animal facility and the areas where infectious materials and/or animals are housed or are manipulated. All persons including facility personnel, service workers, and visitors are advised of the potential hazards (natural or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.
- Protective laboratory coats, gowns or uniforms are recommended to prevent contamination of personal clothing. Gloves are worn to prevent skin contact with contaminated, infectious and hazardous materials, and when handling animals. Double-glove practices should be used when dictated by risk assessment.
- Gloves and personal protective equipment should be removed in a manner that minimizes transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated. Persons must wash their hands after removing gloves and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Eye and face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.

## **ii Special practices (in addition to those listed for ABSL2)**

- All procedures involving the manipulation of infectious materials, handling infected animals or the generation of aerosols must be conducted within a biosafety cabinet or other physical containment device when practical. When a procedure cannot be performed within a biosafety cabinet, a combination of personal protective equipment and other containment devices must be used. Consideration should be given to the use of restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint devices, chemical restraint medications, etc.).
- The risk of infectious aerosols from infected animals or their bedding also can be reduced if animals are housed in containment caging systems (such as solid wall and bottom cages covered with filter bonnets, open cages placed in inward flow ventilated enclosures, HEPA-filter isolators and caging systems, or other equivalent primary containment systems).
- Actively ventilated caging systems must be designed to prevent the escape of microorganisms from the cage. Exhaust plenums for these systems should be sealed to prevent escape of microorganisms if the ventilation system becomes static, and the exhaust must be HEPA filtered. Safety mechanisms should be in place that prevent the cages

and exhaust plenums from becoming positive to the surrounding area should the exhaust fan fail. The system should also be alarmed to indicate when operational malfunctions occur.

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

### **iii Safety equipment**

- Properly maintained biological safety cabinets and other physical containment devices or equipment, should be used for all manipulations of infectious materials and when possible, animals. These manipulations include necropsy, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals.
- The risk of infectious aerosols from infected animals or bedding can be reduced through the use of primary barrier systems. These systems may include solid wall and bottom cages covered with filter bonnets; ventilated cage rack systems; or for larger cages placed in inward flow ventilated enclosures or other equivalent systems or devices.
- A risk assessment should determine the appropriate type of personal protective equipment to be utilized. Protective clothing such as scrub suits or uniforms is worn by personnel within the animal facility. Reusable clothing is appropriately contained and decontaminated before being laundered. Laboratory and protective clothing should never be taken home. Disposable personal protective equipment such as non-woven olefin cover-all suits, wrap-around or solid-front gowns should be worn over this clothing, before entering the areas where infectious materials and/or animals are housed or manipulated. Front-button laboratory coats are unsuitable.
- Disposable personal protective equipment must be removed when leaving the areas where infectious materials and/or animals are housed or are manipulated. Scrub suits and uniforms are removed before leaving the animal facility. Disposable personal protective equipment

and other contaminated waste are appropriately contained and decontaminated prior to disposal.

- Appropriate eye, face and respiratory protection are worn by all personnel entering areas where infectious materials and/or animals are housed or are manipulated. To prevent cross contamination boots, shoe covers, or other protective footwear, are used where indicated.
- Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates.
- Gloves should be worn to protect hands from exposure to hazardous materials. A risk assessment should be performed to identify the appropriate glove for the task and alternatives to latex gloves should be available. Procedures may require the use of wearing two pairs of gloves (double-glove). Gloves are changed when contaminated, integrity has been compromised, or when otherwise necessary. Gloves must not be worn outside of the animal rooms.
- Gloves and personal protective equipment should be removed in a manner that prohibits transfer of infectious materials. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste.
- Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Hand washing should occur after the removal of gloves.

#### **iv Animal facilities (in addition to those listed for ABSL1 and 2)**

- The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking.
- Access to the animal facility is restricted. Doors to areas where infectious materials and/or animals are housed open inward, are self-closing, are kept closed when experimental animals are present, and should never be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.
- Entry into the containment area is via a double-door entry which constitutes an anteroom/airlock and a change room. Showers may be considered based on risk assessment. An additional double-door access anteroom or double-ended “pass-through” autoclave may be provided for movement of supplies and wastes into and out of the facility.
- A hand washing sink is located at the exit of the areas where infectious materials and/or animals are housed or are manipulated. Additional sinks for hand washing should be located in other appropriate locations within the facility. The sink should be hands-free or automatically operated. If the animal facility has multiple segregated areas where infectious materials and/or animals are housed or are manipulated, a

sink must also be available for hand washing at the exit from each segregated area.

- Sink traps are filled with water, and/or appropriate liquid to prevent the migration of vermin and gases.
- The animal facility is designed, constructed, and maintained to facilitate cleaning, decontamination and housekeeping. The interior surfaces (walls, floors, and ceilings) are water resistant. Penetrations in floors, walls and ceiling surfaces are sealed, to included openings around ducts, doors and door frames, to facilitate pest control, proper cleaning, and decontamination. Walls, floors and ceilings should form a sealed and sanitizable surface.
- Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Flooring is seamless, sealed resilient or poured floors with integral cove bases.
- Decontamination of an entire animal room should be considered when there has been gross contamination of the space, significant changes in usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the animal room must be based on the risk assessment.
- External windows are not recommended; if present, windows must be sealed and must be resistant to breakage. The presence of windows may impact facility security and therefore should be assessed by security personnel.
- Ventilation should be provided in accordance with the Guide for Care and Use of Laboratory Animals. The direction of airflow into the animal facility is inward; animal rooms should maintain inward directional airflow compared to adjoining hallways. A ducted exhaust air ventilation system is provided. Exhaust air is discharged to the outside without being recirculated to other rooms. This system creates directional airflow which draws air into the animal room from “clean” areas and toward “contaminated” areas.
- Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process. Filtration and other treatments of the exhaust air may not be required, but should be considered based on site requirements, specific agent manipulations and use conditions. The exhaust must be dispersed away from occupied areas and air intakes, or the exhaust must be HEPA-filtered.
- Personnel must verify that the direction of the airflow (into the animal areas) is proper. It is recommended that a visual monitoring device that indicates and confirms directional inward airflow be provided at the animal room entry. Consideration should be given to installing an HVAC control system to prevent sustained positive pressurization of the animal spaces. Audible alarms should be considered to notify personnel of ventilation and HVAC system failure.
- Floor drains must be maintained and filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.

- Cages are washed in a mechanical cage washer. The mechanical cage washer has a final rinse temperature of at least 180°F. Cages should be autoclaved or otherwise decontaminated prior to removal from ABSL-3 space. The cage wash facility should be designed and constructed to accommodate high pressure spray systems, humidity, strong chemical disinfectants and 180°F water temperatures, during the cage/equipment cleaning process.
- BSCs (Class II, Class III) must be installed so that fluctuations of the room air supply and exhaust do not interfere with its proper operations. Class II BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be recertified at least annually to assure correct performance. Class III BSCs must supply air in such a manner that prevents positive pressurization of the cabinet or the laboratory room.
- All BSCs should be used according to manufacturer's recommendations. When applicable, equipment that may produce infectious aerosols must be contained in devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the animal facility. These HEPA filters should be tested and/or replaced at least annually.
- An autoclave is available which is convenient to the animal rooms where the biohazard is contained. The autoclave is utilized to decontaminate infectious materials and waste before moving it to the outer areas of the facility. If not convenient to areas where infectious materials and/or animals are housed or are manipulated, special practices should be developed for transport of infectious materials designated alternate location(s) within the facility.
- The ABSL 3 facility design and operational procedures must be documented. The facility must be tested to verify that the design and operational parameters have been met prior to use. Facilities should be re-verified at least annually against these procedures as modified by operational experience.
- Additional environmental protection (e.g., personnel showers, HEPA filtration of exhaust air, containment of other piped services, and the provision or effluent decontamination) should be considered if recommended by the agent summary statement, as determined by risk assessment of the site conditions, or other applicable federal, state or local regulations.

#### **d. Invertebrate animal biosafety**

"Arthropod Containment Guidelines" were published in Vector Borne and Zoonotic Diseases and are referenced in the [BMBL](#), 5th Edition, 2009, U.S. Department of Health

and Human Services. Sections of the guidelines specifically mentioned in the BMBL include:

- The Principles of Risk Assessment that discusses arthropods in the context of those known to contain a pathogenic agent, those with uncertain pathogens and those with no agent.
- Biological containment as a significant factor that reduces the hazards associated with accidental escape of arthropods.
- Epidemiological context altering the risks of an escape and its impact on the location/site in which work is performed.
- Genetically modified arthropods with an emphasis on phenotypic change.
- Four arthropod containment levels (ACL 1-4) which follow similar form to the biosafety and animal biosafety levels addressed in the BMBL5, i.e., standard practices, special practices, equipment and facilities.

Certain criteria have also been set forth by the WHO (World Health Organization) regarding arthropod use in research experiments, which includes:

- Separate rooms be provided for infected and non-infected invertebrates.
- The rooms should be capable of being sealed for fumigation.
- Insecticide sprays should be readily available.
- “Chilling” facilities should be provided to reduce, where necessary, the activity of invertebrates.
- Access should be through an anteroom containing insect traps and with arthropod-proof screens on the doors.
- All exhaust ventilation ducts and windows (if operable) should be fitted with arthropod-proof screens.
- Waste traps on sinks and sluices should not be allowed to dry out.
- All waste should be decontaminated by autoclaving, as some invertebrates are not killed by all disinfectants.
- A check should be kept on the numbers of larval and adult forms of flying, crawling and jumping arthropods.
- Containers for ticks and mites should stand in trays of oil.
- Infected or potentially infected flying insects must be contained in double-netted cages.
- Infected or potentially infected arthropods must be handled in biological safety cabinets or isolators.
- Infected or potentially infected arthropods may be manipulated on cooling trays.

Additional references for arthropod containment and facility design include:

1. The American Committee of Medical Entomology of the American Society of Tropical Medicine and Hygiene. [Arthropod Containment Guidelines](#) Version 3.2.

2. The Subcommittee on arthropod-borne viruses. Laboratory safety for arboviruses and certain other viruses of vertebrates. *American Journal of Tropical Medicine and Hygiene*, 1980, 29:1359-1381.
3. National Research Council. Occupational health and safety in the care and use of research animals. Washington, DC, National Academy Press, 1997.
4. Richmond JY, Quimby F. Considerations for working safely with infectious disease agents in research animals. In: Zak O, Sande MA, eds. *Handbook of animal models of infection*. London, Academic Press, 1999:69-74

If you have any questions regarding vertebrate or invertebrate biosafety levels, please call the Biosafety Officer at 335-9553.

## VII Disinfection and Sterilization

Routine cleaning of the laboratory is required to maintain a sanitary, safe working environment. Several different means of decontamination exist and the method you choose will depend on the type of experimental work and the nature of the infectious agent(s) present. Of note, prions are highly resistant to inactivation by most physical and chemical agents; decontamination of prions will be addressed separately in this chapter. Standard procedures should be established for each laboratory, designed to meet the needs of the various levels of biohazards found in your particular lab.

### a. Definitions

Word	Definition
Antimicrobial	An agent that kills microorganisms or suppresses their growth and multiplication.
Antiseptic	A substance that inhibits the growth and development of microorganisms without necessarily killing them. Antiseptics are usually applied on body surfaces.
Biocide	A general term for any agent that kills unicellular and multicellular organisms.
Chemical germicide	A chemical or a mixture of chemicals used to kill microorganisms.
Decontamination	Any process for removing and/or killing microorganisms. The same term is also used for removing or neutralizing hazardous chemicals and radioactive materials.
Disinfectant	A chemical or mixture of chemicals used to kill microorganisms, but not necessarily their spores. Disinfectants are usually applied on inanimate surfaces or objects.
Disinfection	A physical or chemical means of killing microorganisms, but not necessarily their spores.
Microbicide	A chemical or mixture of chemicals that kills microorganisms. The term is often used in place of “biocide”, “germicide” or “antimicrobial”.
Sterilization	A process that destroys and/or removes all classes of microorganisms and their spores.

## **b. Disinfection**

Disinfection is used to eliminate the infectious or pathogenic agent(s) present. A disinfectant is selected according to the specific infectious agent known or suspected to be present, as each chemical compound has a selective germicidal activity. Liquid disinfectants are available under a wide variety of trade names. The more active a compound is, the more likely it is to have undesirable characteristics such as corrosivity. No liquid disinfectant is equally useful or effective under all conditions and for all viable agents. The most practical use of liquid disinfectants is for surface decontamination. At sufficient concentrations they can be used as decontaminants for liquid wastes prior to disposal in the sanitary sewer.

### **i Alcohols**

Alcohols (ethyl and isopropyl) are a good general disinfectant at concentrations of 70-80 percent (volume/volume) in water. They are effective against vegetative bacteria, fungi and lipid-containing viruses but not against spores. The activity against non-lipid viruses varies. The contact time to achieve effective disinfection is at least 10 seconds on skin and at least 3 minutes on environmental surfaces. An advantage of alcohols is that they do not leave any residue on treated items.

Note: Alcohols are volatile and flammable and must not be used near an open flame. Working solutions must be stored properly in order to prevent the evaporation of alcohols.

### **ii Chlorine compounds**

Chlorine is a broad-spectrum germicide and is the recommended general all-purpose laboratory disinfectant. Chlorine is effective against bacteria, mycobacteria, viruses and fungal spores. However, not all bacterial spores are killed by chlorine and the amount of available chlorine must be considered when preparing the disinfectant. A concentration of 5,000 parts per million (ppm) available chlorine is recommended as an all-purpose disinfectant. A higher concentration, near 10,000 ppm available chlorine, is recommended for biohazardous spills, emergency situations involving viruses and in the presence of large amounts of organic matter (protein, including dirt).

Sodium hypochlorite (NaOCl), as an aqueous solution, is sold as bleach. Household commercial bleach contains 5.25 percent available chlorine; solutions of 10 percent or 20 percent will yield concentrations of 5,000 ppm and 10,000 ppm available chlorine, respectively. The activity of chlorine, especially as bleach, is reduced in the presence of protein. Solutions receiving material containing high levels of organic matter several times a day should be replaced

daily, while less frequently used solutions can last for one week. Furthermore, low levels of chlorine gas are naturally released from stored solutions of chlorine and reduce the germicidal activity.

Note: Chlorine gas is highly toxic and therefore bleach should not be mixed with acids which would cause the release of chlorine gas. Additionally, chlorine is highly alkaline and is corrosive to metal. By-products of chlorine can be harmful to humans and the environment, therefore chlorine containing compounds should not be used indiscriminately.

Chloramines release chlorine at slower rates than hypochlorites and therefore higher concentrations are required to achieve equivalent activity to those of hypochlorites. However, chloramine solutions are virtually odor-free and are not inactivated by organic matter to the same extent as hypochlorites. Concentrations of 20,000ppm available chlorine are recommended for both “clean” and “dirty” situations.

### **iii Formaldehyde**

Formaldehyde (HCHO) is effective against vegetative bacteria, spores and viruses. Formaldehyde is available in two forms: as a solid polymer, paraformaldehyde, or as a solution of the gas dissolved in water, formalin. Concentrations of 5-8 percent formalin in water are an effective liquid disinfectant. Formaldehyde - Alcohol solutions of 8 percent formaldehyde in 70 percent alcohol are considered very good for disinfection purposes because of the effectiveness against vegetative bacteria, spores and viruses.

Note: Formaldehyde is carcinogenic and its fumes irritate the eyes and mucous membranes. All storage and use of formaldehyde must be done in a fume hood or well-ventilated area.

### **iv Glutaraldehyde**

Glutaraldehyde (OHC(CH<sub>2</sub>)<sub>3</sub>CHO) is also effective against vegetative bacteria, spores and viruses. It is non-corrosive and faster acting than formaldehyde; however, it takes several hours to kill the bacterial spores. Glutaraldehyde is often purchased as a 2 percent solution and requires “activation” (made alkaline) before use by adding a bicarbonate compound supplied with the product. A solution of glutaraldehyde which has become turbid should not be used.

Note: Glutaraldehyde is toxic and its fumes irritate the eyes and mucous membranes. All use must be done in a fume hood or well-ventilated area.

Additionally, it is not recommended as a spray or solution to decontaminate environmental surfaces.

**v Hydrogen peroxide and peracids**

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and peracids are strong oxidants and are active against vegetative bacteria, spores and viruses. They are safer than chlorine to both humans and the environment. Hydrogen peroxide is available as a 3 percent ready-to-use solution or as a 30 percent aqueous solution that should be diluted 5-10 times with sterilized water before use; however, 3-6 percent solutions are slow acting and limited as germicides. Hydrogen peroxide can be used on work surfaces of laboratory benches and biosafety cabinets; stronger solutions can be used to disinfect heat-sensitive medical/dental devices.

Note: Hydrogen peroxide and peracids are corrosive to metals, including aluminium, copper, brass and zinc, and can decolourize fabrics, hair, skin and mucous membranes. Items treated with them must be thoroughly rinsed before contact with eyes and mucous membranes.

**vi Iodine and iodophors**

Iodine and iodophors are effective against lipid-containing viruses, bacteria and fungi but exhibit variable activity against mycobacteria, non-enveloped viruses and bacterial spores. Iodine can stain fabrics and environmental surfaces, is neutralized by organic matter and is generally unsuitable for use as a disinfectant. However, iodophors and tinctures of iodine are good antiseptics. Several advantages of iodophors include:

- a wide spectrum of anti-microbial and antiviral activity
- a built-in indicator (if the solution is brown or yellow, it is still active)
- use as a preoperative skin antiseptic and surgical scrub

Note: Iodine can be toxic and antiseptics based on iodine are generally unsuitable for use on medical/dental devices. Iodine should not be used on aluminum or copper.

**vii Mercurials**

Mercurials are toxic and therefore are not recommended for use.

**viii Phenolic compounds**

Phenolic compounds are active against vegetative bacteria (including mycobacteria), fungi and lipid-containing viruses. They are not active against bacterial spores and show variable use against non-lipid viruses. Phenolic compounds are commonly used as antiseptics (e.g., triclosan and chloroxynol); however, in laboratory-based studies, bacteria made resistant to low concentrations of triclosan also show resistance to certain types of antibiotics.

Note: Phenolic compounds are not recommended for use on food contact surfaces and around areas with young children. They can be absorbed by rubber and penetrate the skin. Often, they have an unpleasant odor (e.g., Amphyl, Vesphene II).

## **ix Quaternary ammonium compounds**

Quaternary ammonium compounds are active against vegetative bacteria and non-lipid containing viruses but not against bacterial spores at the usual concentrations (1:750). These compounds are often used as mixtures and in combination with other germicides. However, the activity of quaternary ammonium compounds may be neutralized by organic matter, water hardness and anionic detergents (soap).

Note: Potentially harmful bacteria can grow in quaternary ammonium compound solutions. Also, these compounds may accumulate in the environment as a result of low biodegradability.

## **c. General procedures**

The goal of decontamination is to protect personnel and the environment from exposure to biological agents and to prevent contamination of experimental materials. Outlined below are several general guidelines that should be followed in the research laboratory.

- Infectious materials and contaminated equipment originating from the lab should be sterilized before being washed and stored or discarded. Autoclaving is the preferred method. Each individual working with infectious material is responsible for its sterilization.
- Biohazardous materials should not be placed in autoclaves overnight in anticipation of autoclaving the next day. To minimize hazard to firefighters or disaster crews, all biohazardous materials should be placed in an appropriately marked refrigerator or incubator, sterilized or otherwise confined at the close of each work day.
- Special precautions should be taken to prevent accidental removal of material from an autoclave before it has been sterilized or the simultaneous opening of both doors on a double-ended autoclave.
- Dry hypochlorites or any other strong oxidizing material must not be autoclaved with organic materials such as paper, cloth, or oil. Oxidizer + organic material + heat = explosion potential.
- Laboratory rooms containing biohazardous materials should designate, where appropriate, separate areas or containers labeled:
  - BIOHAZARDOUS--TO BE AUTOCLAVED
  - OR

- NON-INFECTIOUS--TO BE CLEANED
- All floors, laboratory benches and other surfaces in buildings where biohazardous materials are handled should be disinfected as often as deemed necessary by the supervisor. After completion of operations involving plating, pipetting, centrifuging and similar procedures with biohazardous materials, the surroundings should be disinfected.
- Floor drains should be flooded with water at least once each week or filled with mineral oil in order to fill traps and prevent backflow of sewer gases.
- Floors should be wet mopped and a disinfectant added to the wash. Vacuum cleaners equipped with HEPA filtration may be used.
- Stock solutions of suitable disinfectants should be maintained in each laboratory for disinfection purposes

#### **d. Local environmental decontamination**

Surfaces can be decontaminated using a solution of bleach; a solution containing 5,000 ppm available chlorine may be suitable for general environmental sanitation, but stronger solutions (10,000 ppm) are recommended when dealing with high-risk situations. For environmental decontamination, formulated solutions containing 3 percent hydrogen peroxide make suitable substitutes for bleach solutions.

Fumigation of spaces with a vaporous solution of hydrogen peroxide is an effective decontamination method and is replacing formaldehyde gas decontaminations. Relatively low concentrations of hydrogen peroxide (1 milligram/liter) can be effective area fumigants. As with any disinfectant, the efficacy of hydrogen peroxide will vary depending on the contaminating organism, the presence of organic and/or inorganic material and on the type of surface being decontaminated. One advantage over paraformaldehyde is that the residual vapor of hydrogen peroxide decomposes into water and oxygen. Contact EHS's Biosafety Office if you have any questions or concerns.

#### **e. Decontamination of BSCs**

Class II BSCs can be decontaminated with vaporized hydrogen peroxide. This procedure shall only be done by qualified technicians due to the potential for exposure to biohazardous agents and the chemicals used. Cabinets must be decontaminated before they are moved, before any repairs are done that require access to the plenum and before the filters are changed. The Biosafety Specialist, should be contacted ([bio-cabinet@uiowa.edu](mailto:bio-cabinet@uiowa.edu) or 335-7702) when a BSC decontamination is required or for any questions regarding decontamination of BSCs.

#### **f. Hand washing**

Whenever possible, appropriate gloves should be worn when handling biohazardous materials (see [Table 3](#)). However, gloves do not eliminate the need for regular and proper hand washing. Hands must be washed with soap and running water after handling biohazardous materials and animals, before leaving the laboratory and before eating. In most situations, thorough washing of hands with ordinary soap and water is

sufficient to decontaminate them, but the use of germicidal soaps is recommended in high-risk situations. Alcohol-based hand-rubs should be used to decontaminate lightly soiled hands when proper hand washing is not available.

## **g. Heat disinfection and sterilization**

Heat is the most common physical agent used for the decontamination of pathogens. “Dry” heat, which is totally non-corrosive, is used to process many items of laboratory ware which can withstand temperatures of 160°C or higher for 2-4 hours. Burning or incineration (see below) is also a form of dry heat. “Moist” heat is most effective when used in the form of autoclaving. Boiling does not necessarily kill all microorganisms and/or pathogens, but it may be used as the minimum processing for disinfection where other methods (chemical disinfection or decontamination, autoclaving) are not applicable or available.

Sterilized items must be handled and stored such that they remain uncontaminated until used.

The necessary treatment to achieve sterility will vary in relation to the volume of material treated, its contamination level, moisture content and other factors. The material presented below is to be used as general criteria for biohazardous/infectious agents coming from BSL2 laboratories. These materials are all processed in the medical waste incinerator, but must be handled several times during transport. Proper containment and treatment at the source reduces the potential for an accidental exposure.

### **i Autoclaving**

Autoclaving is the most effective and reliable means of sterilizing laboratory materials. Autoclaving sterilizes material using saturated steam under pressure. Standard autoclave cycles for commonly used material are shown below.

- Laundry: 121°C for 30 minutes with 15 minutes prevacuum of 27 inches of mercury (in. Hg)
- Glassware and trash: 121°C for 1 hour with 15 minutes prevacuum of 27 in. Hg
- Liquids: 121°C for 1 hour for each gallon

### **ii General autoclaving guidelines**

- Every autoclave and sterilizer should be inspected and serviced on a regular basis. This will help ensure the equipment is functioning properly.
- Each unit should have a standard operating procedure written in sufficient detail to ensure that operators will use the equipment properly.

- Units should be tested regularly with a commercial preparation of *Bacillus stearothermophilus* (a biological indicator), in particular, any unit in a BSL3 facility.
- Tape indicators (autoclave tape) with heat sensitive, chemical indicators should be used in every autoclave load. Please note: the indicators only verify that the autoclave has reached normal operating temperatures; they have no time factor. Therefore, tape indicators cannot be used to prove organisms are actually killed during an autoclave run.
- Keep detailed records on biological tests, recording thermometers, and service work performed on the unit.
- Do not autoclave flammable liquids, toxic chemicals, carcinogens, cytotoxic drugs, or radioactive materials. The careless autoclaving of hazardous materials may generate toxic vapors or explosive environments.
- Do not autoclave items containing more than trace amounts of solvents and other volatile chemicals or corrosives (e.g., phenol, trichloroacetic acid, ether, chloroform, hypochlorite).
- Do not autoclave bulk liquids without following the manufacturer's written instructions. See the following section on autoclave containers for more information.
- Neutralize waste containing bleach with equal amounts of 1 percent sodium thiosulfate in water prior to autoclaving.
- High density wastes or materials that insulate the agents from heat and steam penetration are not suitable for steam sterilization. Items that are covered with dirt or film require additional retention times. The importance of properly cleaning items to be sterilized cannot be over emphasized.
- Place all autoclaved infectious waste into red bags for disposal.

### **iii Autoclave bags / container guidelines**

- The proper packaging and containment of infectious materials are crucial to achieve effective sterilization. The most frequent reason for sterilization failure is the lack of contact between the steam and microorganisms.
- To facilitate steam penetration, bottle caps and stoppers should be loosened after placement into the chamber. If left sealed, they may not be properly sterilized and could burst violently if exposed to extreme heat.
- Most bags that are marketed as autoclavable are not suitable if closed because the steam will not penetrate them. Steam resistant bags must be left open or have holes punched into the top to allow the steam to penetrate. Do not transfer open bags to the autoclave.
- Never close autoclave bags that have a printed warning stating they are to remain open during sterilization. If air remains trapped in the bag, the material may not be properly sterilized.

- Autoclave bags that allow steam penetration tend to melt or crumble during the sterilization process. Autoclavable bags may be placed inside paper bags, or open steam resistant polypropylene bags.
- Autoclavable bags can leak so they should be placed into a shallow stainless steel pan. Plastic pans are less effective because they do not transfer heat as fast or efficiently.
- Sterilization of bulk liquids requires special care to prevent the containers from exploding.
- Each gallon of infectious liquid must be autoclaved for one hour at 250°F at 15 pounds per square inch. Closures and lids must be loosened prior to sterilizing.
- Bulk solutions must be sterilized separately from all other items in a load dedicated to liquids only. Solutions are subjected to a cycle designed specifically for liquids.
- Sterilized liquids must be allowed to cool before unloading. Removing hot bottles may cause them to explode.

#### **iv Loading the autoclave**

- Follow the manufacturer’s instructions before attempting to load the chamber.
- Materials should be loosely packed in the chamber for easy steam penetration and air removal. Add 250ml of water to solid waste in order to create additional steam that drives residual air from the bag.
- Transfer infectious waste to the autoclave in a sealed secondary container. The autoclave bags should be left open during autoclaving to insure steam penetration and sufficient temperatures inside the bag are achieved.
- Avoid rough handling of waste containers in order to minimize the formation of infectious aerosols.

#### **v Unloading the autoclave**

- Follow the manufacturer’s instructions before unloading the chamber.
- Do not open the autoclave while the chamber is pressurized, released steam can cause severe burns.
- Wear heat-resistant gloves, safety glasses/goggles and a laboratory coat when removing items.
- Wait until the autoclave has cooled prior to opening the door. Most autoclaves have safety interlocks that prevent the door from opening when the temperature inside is greater than 80°C; however, a puff of steam may be ejected if the autoclave is opened immediately after the cycle.
- Avoid standing directly in front of the autoclave door when it is opened after a run.
- Handle waste containers containing liquids with care to avoid being burned by hot liquid splashes or spills. Liquids should be allowed to cool

for 20 minutes before transport to prevent sudden eruption from the containment vessel.

## **h. Decontamination of prion-containing material**

Prions, also referred to as “unconventional” infectious agents or “agents of transmissible spongiform encephalopathies,” are comprised of protein only. They can cause Creutzfeldt-Jakob disease in humans, scrapie in sheep, bovine spongiform encephalopathy in cattle, etc. These infectious agents are unusually resistant to inactivation by the normal processes of laboratory disinfection and sterilization and materials suspected of containing them require special processing before reuse or disposal. Therefore, it is important to stress the use of disposable instruments whenever possible and the use of a disposable protective covering for the work surface of a BSC when working with materials known to contain or suspected to contain prions.

Laboratory transmission of prions can occur through ingestion of contaminated materials or puncture of the researcher’s skin. The following precautions should be taken when working with prions or material suspected of containing prions:

- Use dedicated equipment, e.g., equipment not shared with other laboratories.
- Wear disposable laboratory protective clothing (gowns, aprons) and gloves (steel mesh gloves between rubber gloves for pathologists).
- Use disposable plastic ware that can be treated and discarded as dry waste.
- Do not use tissue processors due to the problems with disinfection; jars and beakers should be used instead.
- Perform all manipulations in a BSC.
- Take great care to avoid aerosol production, ingestion, and cuts and punctures of the skin.
- Regard formalin-fixed cultures as still infectious, even after prolonged exposure to formalin.

To date, the World Health Organization suggests the following for the inactivation of prions:

- All non-disposable instruments, including steel mesh gloves, must be collected for decontamination.
- Infectious liquid waste should be treated with sodium hypochlorite containing available chlorine at 20 gram/liter (2%) (final concentration) for one hour.
- Bench waste, including disposable gloves, gowns, and aprons, should be autoclaved using a porous load steam sterilizer at 134-137°C for a single cycle of 18 minutes, or six successive cycles of 3 minutes each, followed by incineration.
- Instruments that cannot be autoclaved can be cleaned by repeated wetting with sodium hypochlorite containing available chlorine at 20 gram/liter (2%) over a one hour period. Washing to remove residual sodium hypochlorite is required.

- Instruments that can be autoclaved should be soaked in sodium hypochlorite containing available chlorine at 20 gram/liter (2%) for one hour and the rinsed well in water before autoclaving.
- Contaminated BSC and other surfaces can be decontaminated with sodium hypochlorite containing available chlorine at 20 gram/liter (2%) for one hour.
- Paraformaldehyde vaporization procedures do not diminish prion titres and prions are resistant to ultraviolet irradiation. However, BSCs must continue to be decontaminated by standard methods to inactivate other agents that may be present. Surface decontamination of BSCs should be done with 1N NaOH as discussed below.
- HEPA filters should be incinerated at a minimum temperature of 1000°C after removal. Recommended additional steps prior to incineration include:
  - Spraying the exposed face of the filter with lacquer hairspray prior to removal
  - “Bagging” of filters during removal
  - Removal of the HEPA filter from the working chamber so that the inaccessible plenum of the cabinet is not contaminated
- Histological samples containing prions are substantially inactivated after exposure to 96% formic acid for one hour.

The BMBL, 5th Edition, suggests the following prion inactivation methods:

- For reusable instruments and surfaces immerse in 1N NaOH or sodium hypochlorite (2% free chlorine concentration) for 1 hour followed by steam autoclaving at 121°C for 1 hour. Clean and sterilize by conventional means.
- Dry waste, animal carcasses and other tissue should be incinerated with a minimum secondary temperature of 1000°C.
- Work surfaces of BSCs must be decontaminated with 1N NaOH and rinsed with water.
- HEPA filters should be bagged out and incinerated.

## **i. Spills**

Each laboratory must have appropriate equipment and materials and develop procedures for dealing with spills. Spill procedure charts are available from EHS and should be displayed in a prominent position in the laboratory and also included with the spill kit. Each laboratory should have a spill kit which is clearly labeled; it is recommended that a sign be posted indicating where the kit is located (such as with an arrow) if the kit is placed up on a shelf or under a cabinet for example. A basic biological spill kit could include the following:

- Protective clothing, e.g., lab coat, gloves and face protection (i.e. face shield, safety glasses or goggles)
- Scoops and/or autoclavable dustpan
- Forceps for picking up broken glass
- absorbent material (i.e. paper towels)
- Concentrated disinfectant (chlorine bleach or other appropriate disinfectant)

- Nonflammable detergent
- Biohazard bag

Chemicals used in the laboratory will require additional items dependent on the chemical in use; see the section on Spill and Emergency Plans within the Chemical Hygiene Plan.

The potential health risk of the spilled agent must be considered. For example, with *Mycobacterium tuberculosis* the risk of exposure from the spill of a small quantity might be many times that of a much larger spill of *E. coli*. A minimally biohazardous material (BSL1 or RG1 agent) spilled without generating significant aerosols may be cleaned up with a paper towel soaked in an effective decontaminating agent. A spill of a large volume with generation of aerosols will require personnel to wear protective clothing and possibly respiratory protection, depending on the biological agent involved.

## **i Spills Inside a BSC**

### *Preparation*

Cleanup materials should be kept in the cabinet so they are available when a spill occurs.

### *Cleanup*

- Do not remove anything from the cabinet, including your hands, to prevent dispersion outside the cabinet.
- Continue to operate the cabinet to clear the air of contaminants.
- While wearing gloves, use a clean cloth and appropriate disinfectant solution (most commonly used: freshly-prepared 10% dilution of household bleach) to:
  - Clean up the spill
  - Disinfect interior surfaces of the cabinet, including walls and work surfaces
  - Wipe down any equipment contained within the cabinet
  - In moderate to high-risk spills, flood catch-basins
- Prevent the generation and escape of aerosols and contaminants from the cabinet during decontamination by working cautiously to prevent splashing materials outside the cabinet.
- Allow a 20-minute disinfectant contact period.
- To prevent corrosion of the cabinet's stainless steel surfaces, wipe disinfected surfaces with 70% alcohol to remove the bleach.
- Put all cleanup materials in a biohazard bag (before removing from the area) and dispose of as biohazardous waste.
- Wash hands and any exposed skin with soap and water.

- Allow the cabinet to run for at least 10 minutes following cleanup prior to using again.

## ii Spills Outside a Biological Safety Cabinet

### *BSL1 / RG1 Agent or "Small spill" of a BSL2 agent*

A spill is considered to be small if it is easily contained, has not generated infectious aerosols, and is not considered to be a significant threat to the personnel in other areas of the building.

- Wear disposable gloves and a lab coat.
- Soak paper towels in disinfectant and place over the spill area, allowing sufficient contact time with the disinfectant (20 minutes- most commonly used: freshly-prepared 10% dilution of household bleach).
- Clean spill area with fresh towels soaked in disinfectant.
- Pick up any broken glass with forceps and place in a sharps container.
- Put all materials used in the cleanup into a biohazard bag and dispose of as biohazardous waste.
- Thoroughly wash hands with soap and water.

### *BSL2 / RG2 Agent or "Large Spill"*

A spill is considered to be large if it is difficult to contain within the laboratory or the facility, and/or it constitutes a significant health hazard. This type of spill may require special assistance in controlling and clean up.

#### Preparation:

- Evacuate the area immediately.
- If biological safety cabinet or fume hood is in the room, leave it on and immediately exit the room. Close and lock the door.
- Post a "Biohazard" and "Do Not Enter" sign on the door to keep people out of the area and to prevent the spread of the contaminant.
- Notify supervisor and EHS's Biological Safety Section

#### Cleanup:

- Thoroughly wash face, hands and any exposed body area. Remove all contaminated clothing, and decontaminate (autoclave, if necessary). If necessary, use an emergency shower in the immediate area.
- Allow at least 30 minutes for droplets to settle and aerosols to be reduced before reentering.
- Don protective equipment (long sleeved lab coat, disposable gloves, safety goggles and face shield and disposable shoe covers, if needed).

- If the spill is large, apply absorbent booms or dike around the spill area to avoid spreading.
- Cover spill area with paper towels or absorbent pads soaked in disinfectant.
- Decontaminate with an appropriate disinfectant. Pour the disinfectant slowly around the spill, not on the spill, to avoid aerosolizing the material.
- Allow a 20-minute disinfectant contact period.
- Wipe down all surfaces that may have been splashed.
- Clean up the liquid working from the outside of the spill area inward to avoid spreading the spill.
- Using an autoclavable dust pan and squeegee, transfer all contaminated glassware or sharp material into a sharp's bucket.
- Re-wipe spill area with disinfectant.
- Dispose of all cleanup items in the proper container and dispose of as biohazardous waste.
- Wash hands and any exposed skin with soap and water.
- For large spills, contact EHS's Biological Safety Section for assistance in determining when additional decontamination is necessary.

#### *Spills – Blood*

- While wearing appropriate PPE, including lab coat and gloves, use paper towels to absorb the spill.
- Cleanup all visible blood with a detergent solution.
- Soak clean paper towels with a disinfectant (freshly-prepared 10% dilution of household bleach) and wipe down spill area.
- Place all cleanup material in a biohazard bag and dispose of as biohazardous waste.
- Wash hands and any exposed skin with soap and water.

### **iii Spills - BSL3 / RG3 Agent**

The Biosafety Office has developed a Biosafety Manual specific to the BSL3 laboratory which addresses biohazardous spills. Please refer to the BSL3 manual for clean-up procedures in the BSL3 facility.

## **j. Disposal**

Biohazardous waste disposal must be handled in accordance with procedures and practices established by the University. This waste must be segregated from general waste at the point of origin. Potentially infectious material or biohazard waste must be discarded directly into red-bag lined Rubbermaid transport containers or a red-bag lined white biohazard box which is clearly identifiable and distinguishable from general waste. Containers must be marked with the universal biohazard symbol ([Figure 1](#)). Plastic bags must be distinctly colored red or orange, and marked with the universal biohazard symbol.

Waste disposal procedures are outlined in the [Waste Disposal Guidelines and Procedures Manual](#) available on EHS's Web site.

## VIII Research Involving Recombinant DNA (rDNA)

As a condition for NIH funding, all rDNA research conducted at or sponsored by the University, irrespective of the source of funding, must comply with the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* ([NIH Guidelines](#)). The *NIH Guidelines* defines rDNA as either (1) molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell or (2) molecules that result from the replication of those described in (1) above. Non-compliance by a PI may result in (1) suspension, limitation or termination of financial assistance for the noncompliant NIH-funded research project and of NIH funds for other rDNA research at the institution or (2) a requirement for prior NIH approval of any or all recombinant DNA projects at the University. In order to assure compliance, all PIs working with rDNA need to be familiar with the latest edition of the *NIH Guidelines*; an [online course](#), *NIH Guidelines for Research Involving Recombinant DNA*, offered through EHS, has been developed to assist PIs in meeting this obligation.

An overview of the University's rDNA program is presented in the [Recombinant DNA Procedural Manual](#), which can be accessed using the link provided or by visiting [www.ehs.research.uiowa.edu](http://www.ehs.research.uiowa.edu), locating the "Biological Safety" section on the right hand menu, clicking on "Recombinant DNA" and then clicking "rDNA Procedural Manual".

## IX Select Agents and Toxins

As required by the Public Health Security and Bioterrorism Act of 2002, the DHHS (Department of Health and Human Services) and USDA (United States Department of Agriculture) have set forth rules regarding the possession, use and transfer of select agents and toxins. The biological agents and toxins subject to these rules have the potential to pose a severe threat to public health and safety, to animal health or to animal products. These rules, defined as the Select Agent Program, are outlined in the CFR (Code of Federal Regulations), Title 42, part 73, Possession, Use and Transfer of Select Agents and Toxins. The initial program took effect in 2002; the interim Final Rule has since been replaced with the Final Rule on March 18, 2005.

An overview of the Select Agent Program is presented in the [Select Agents and Toxins Use Manual](#), which can be accessed using the link provided or by visiting [www.ehs.research.uiowa.edu](http://www.ehs.research.uiowa.edu), locating the "Biological Safety" section on the right hand menu, clicking on "Select Agents and Toxins" and then clicking "SAT Manual".

## X Shipping Regulations

IATA (International Air Transport Association) and DOT (Department of Transportation) regulate the shipping of infectious substances and diagnostic specimens. Specific requirements

regarding the classification of agents, package preparation, package marking/identification and shipper's declaration have been established. It is the shipper's responsibility to be aware and to adhere to applicable laws, regulations and requirements.

## **a. Infectious substances**

Infectious substances are substances known to contain, or can reasonably be expected to contain viable microorganisms, including bacteria, viruses, rickettsia, parasites, or fungi, or other agents such as prions, that can cause disease in humans or animals. Cultures or lab stocks (e.g., tissue culture systems and agar plates) in which such pathogens are amplified or propagated in high concentrations are also considered an infectious substance.

Infectious substances are further categorized as Category A and Category B infectious substances.

Category A infectious substances are cultures of microorganisms listed in the far right column of Appendix B of this manual "Microorganism Classified as Category A only when Cultured", or materials (such as tissue or blood, in addition to cultures) that contain or can reasonably be expected to contain organisms listed in the center column of [Appendix B](#).

Category B infectious substances are those that do not meet the criteria for inclusion as a Category A substance. Category B substances should be labeled as "Biological substance, Category B."

A culture plate or slant shipped for diagnosis or clinical purposes is considered a "Biological substance, Category B", unless it is a culture of a highly pathogenic organism or is included in Appendix B (center or far right columns). A patient specimen (blood, tissue, etc.) suspected to contain an organism found in the far right column of Appendix B, should be shipped as a "Biological substance, Category B." For example, a blood or tissue sample from a patient with rabies or HIV would be shipped as a "Biological substance, Category B" since Appendix B lists each of these viruses as "Classified as Category A only when Cultured" (i.e. far right column).

## **b. Patient specimens**

Specimens taken directly from a patient (either human or animal) may be classified as an "Exempt human Specimen" or "Exempt animal specimen", if there is minimal likelihood that the specimen contains a pathogen. The likelihood that the specimen contains a pathogen must be determined by a professional and be based on the patient's medical history, the patient's symptoms, and endemic local conditions.

### **c. Dry ice shipping**

Dry ice is included in the Miscellaneous Hazard Class 9 and as a result, any shipments with dry ice are regulated by DOT/IATA. If the shipment also includes an infectious substance or diagnostic specimen, packing instructions for those agents must be followed and the Infectious Substances/Diagnostic Substances training completed. DOT/IATA Dangerous Goods Regulations require that anyone using dry ice in shipments or anyone signing documentation for a dry ice shipment (e.g., FedEx Air Waybill) must have current shipping training.

### **d. Training**

Current regulations for shipping infectious substances/diagnostic specimens can be found through IATA, DOT, USPS (United States Postal Service), FedEx and by contacting EHS's Biosafety Staff (353-5679 or 335-9553).

EHS offers ICON training [courses](#) for shipping infectious substances and patient specimens with or without dry ice and a separate course for shipments where the only hazard is dry ice. You must complete applicable training and have documentation of such training. Due to changes in shipping regulations made by the DOT/IATA, online training must be completed every two years, or more often, as the regulations change. Any questions should be directed to the Biosafety Staff at EHS.

## **XI Safety Training**

Departments must provide employees with information and training in order to ensure that they are apprised of biohazards in their work area. Training may take the form of individual instruction, group seminars, audiovisual presentations, handout material or any combination of the above. Training should include the specific hazards associated with agents in the work area when generic training is insufficient to address specific hazards. Training should be provided at the time of an employee's initial assignment to a work area where biohazardous agents are present and prior to assignment involving new exposure situations. Employees should receive periodic refresher information and training. All training must be documented.

Information and training provided by departments should include all of the following:

- The location and availability of the written Biological Safety Manual.
- The health hazards, signs and symptoms associated with exposure(s) and infection(s) with the biohazardous agent(s) used in the work area.
- The measures employees can take to protect themselves from these hazards, including specific procedures the University or department has implemented such as appropriate work practices, emergency procedures and personal protective equipment.
- The location and availability of reference material on the hazards, safe handling, storage and disposal of biohazardous agents.

Although students are not covered under IOSH (Iowa's Occupational Safety and Health Administration), they should be aware of biohazards in teaching situations and be provided information and equipment to protect themselves from those hazards. Departments should provide student training at the beginning of each course in which biohazardous agents are used, with specific safety instructions provided at the beginning of each class period.

Departments are responsible for ensuring that their employees and students receive the proper training as stipulated in the Biosafety Manual. Online, web-based courses are currently available for all training courses through EHS's Web site. EHS provides training courses in the following areas:

- Biological Safety
- Chemical Safety
- Ergonomics
- General Safety
- Hazardous Waste Management
- Industrial Hygiene
- Radiation Safety including Laser Safety

## a. Required courses

EHS has compiled a [Safety Training Course Guide](#), listing the training courses offered and the intended audience for each course. Courses that may be required for individuals working in biological laboratories are listed below.

- Basic Biological Safety. This course is required initially and recommended annually for all individuals working in a lab space where biological agents are likely to be present.
- Bloodborne Pathogens. This course is required in order to fulfill OSHA's initial training mandate for all individuals who are identified as at risk for exposure to bloodborne pathogens or other potentially infectious materials. Four different courses are available; the employee and their supervisor should decide which course is appropriate for them.
  - Bloodborne Pathogens, Lab is designed for laboratory personnel and students.
  - Bloodborne Pathogens, Non-lab is designed for non-laboratory staff (i.e. Athletics, Residence Services, and Public Safety).
  - Bloodborne Pathogens for FM, Housing & Dining Employees is designed specifically for staff in Facilities Management and University Housing and Dining.

- Bloodborne Pathogens Refresher. This course is required in order to fulfill OSHA's annual training mandate for all individuals who are identified as at risk for exposure to bloodborne pathogens or other potentially infectious materials. The refresher course may be taken annually only if staff has already completed one of the Bloodborne Pathogens courses listed above, as appropriate.
- Shipping with Dry Ice. This course is required by IATA (International Air Transport Association) and Department of Transportation (DOT) regulations initially and then every two years for individuals who are shipping with dry ice as the only hazardous material.
- Shipping Infectious Substances. This course is required by IATA and DOT regulations initially and then every two years for individuals who are shipping infectious substances and/or diagnostic specimens with or without dry ice.
- Recombinant DNA (rDNA) Research, NIH Guidelines. This course is required for all staff (PIs, research assistants and students) involved in research utilizing recombinant DNA or recombinant organisms.

These courses cover general information and in some cases may not fulfill all training topics required by DOT and/or OSHA. In some cases, site-specific, departmental information must be provided in addition to the completion of EHS courses in order to be in regulatory compliance. Please direct any questions regarding the type of training required by your laboratory to EHS's Biological Safety Staff. Appropriate contact information for each biosafety program is available on the [contact us link](#) on our website.

## **b. Record keeping**

### **i Training Records**

OSHA requires that records of Bloodborne Pathogens safety training be maintained by employers, which means that departments or individual research laboratories must have records available upon request. A site-specific training form is available within the Bloodborne Pathogens training course; the supervisor is responsible for ensuring that site-specific training is completed in a timely manner and that a record of the site-specific training is kept by his or her department.

IATA/DOT require records of Shipping Infectious Substances and Diagnostic Specimens with or without Dry Ice training be maintained for three years and be available upon request. A certificate/training verification record is available within each specific shipping course; the supervisor is responsible for ensuring that any additional training is completed in a timely manner and that all training records are kept by his or her department.

## ii **Medical Records**

Confidential medical records are maintained for employees and students receiving medical surveillance and medical care at the UEHC. It is the responsibility of the employee to ensure that their medical records are kept current.

# XII **Exposure Evaluation and Follow-Up**

## a. **Medical evaluation**

### i **Where to seek medical treatment following an exposure**

In case of a medical emergency, injured workers should proceed to University of Iowa Hospitals and Clinics (UIHC) Emergency Department.

When a hazardous exposure incident occurs, a medical evaluation and follow-up will be done by UI Occupational Health Office-North Liberty, which is located at 3 Lions Drive in North Liberty, IA and can be reached by calling 319-356-3335. For BBP exposures (i.e. exposure to human blood or body fluids), a medical evaluation and follow-up will be done by the University Employee Health Center (UEHC), which is located on the first floor of Boyd tower in the UIHC and can be reached by calling 319-356-3631.

### ii **What to do following an exposure**

1. Cleanse the exposed area thoroughly using mild soap and water. For a mucus membrane exposure, flush the area with copious amounts of water.
2. Report the incident immediately to your supervisor. Refer to the [University Operations Manual](#) (Part III. Human Resources, Chapter 34: Accidents) for the policy.
3. Report to the proper facility for medical evaluation and treatment.
  - a. During working hours (Mon-Fri 8:00-4:30), call UI Occupational Health Office-North Liberty or UEHC for directions and to arrange evaluation and treatment. During non-working hours and holidays, go directly to UIHC Emergency Department; they can be reached at 319-356-2233. If seen in the Emergency Department, contact UEHC or UI Occupational Health Office-North Liberty for a follow-up the next working day.
  - b. During your medical evaluation you should:
    - Inform medical personnel of the agent(s) or material to which you were exposed. If the exposure involved a chemical, provide medical personnel with the chemical's SDS if possible.
    - Inform medical personnel of the conditions and route under which exposure occurred.
    - Discuss with medical personnel the signs and symptoms of infection with the agent(s) to which you were exposed. Ensure you are made aware of the

signs/symptoms to watch for following your visit and what you should do if those signs/symptoms appear.

**iii Supervisor Responsibilities**

A [Worker's Compensation form](#) must be completed online through the Employee Self Service site within 24 hours. As soon as possible, document the exposure route and circumstances of the incident.

**b. Control method evaluation**

EHS's Biosafety Staff, in conjunction with the PI and the employee(s) involved, will evaluate the circumstances of the exposure incident. The goal of this evaluation is to identify and correct problems in order to prevent recurrence of similar incidents. An [Incident Investigation form](#) will be completed during this evaluation.

The evaluation procedure includes:

- Documentation of the route of exposure and circumstances under which the incident occurred.
- Evaluation of the policies and "failures to control" at the time of the incident.
- Recording engineering controls that were in place at the time.
- Recording work practices and protective equipment or clothing that were used at the time of the incident.
- Determining what action(s) could prevent this or a similar incident in the future.

Appendix A: List of additional resources

[Biosafety in Microbiological and Biomedical Laboratories](#) (BMBL), 5th Edition, February 2009, U.S. Department of Health and Human Services Center for Disease Control and Prevention and National Institutes of Health

[Bloodborne Pathogens](#), 29 CFR 1910.1030

[Centers for Disease Control and Prevention](#), Department of Health and Human Services

[Federal Select Agent Program](#), Department of Health and Human Services, Centers for Disease Control and Prevention

[Guidelines for Research Involving Recombinant DNA Molecules](#) (NIH Guidelines), National Institutes of Health

[Laboratory Biosafety Manual](#), 3rd Edition, 2004, World Health Organization

[National Institutes of Health](#)

[Occupational Safety and Health Administration](#) (OSHA), United States Department of Labor

**Appendix B: Indicative Examples of Category A Infectious Substances\***

UN # and Proper Shipping Name	Microorganism Classified as Category A in any Form (Always Classified as Category A)	Microorganism Classified as Category A only when Cultured
<p><b>UN 2814</b></p> <p>Infectious substance, affecting humans</p>	<p>Crimean-Congo hemorrhagic fever virus</p> <p>Ebola virus</p> <p>Flexal virus</p> <p>Guanarito virus</p> <p>Hantaan virus</p> <p>Hantaviruses causing haemorrhagic fever with renal syndrome</p> <p>Hendra virus</p> <p>Junin virus</p> <p>Kyasanur Forest disease virus</p> <p>Lassa virus</p> <p>Machupo virus</p> <p>Marburg virus</p> <p>Monkeypox virus</p> <p>Nipah virus</p> <p>Omsk hemorrhagic fever virus</p> <p>Sabia virus</p> <p>Variola virus</p>	<p><i>Bacillus anthracis</i></p> <p><i>Brucella abortus</i></p> <p><i>Brucella melitensis</i></p> <p><i>Brucella suis</i></p> <p><i>Burkholderia mallei-Pseudomonas mallei</i> - Glanders</p> <p><i>Burkholderia pseudomallei-Pseudomonas pseudomallei</i></p> <p><i>Chlamydia psittaci</i>- avian strains <i>Clostridium botulinum</i></p> <p><i>Coccidioides immitis</i></p> <p><i>Coxiella burnetii</i></p> <p>Dengue virus</p> <p>Eastern equine encephalitis virus</p> <p><i>Escherichia coli</i>, verotoxigenic</p> <p>Far Eastern Tick-borne Encephalitis virus (formally known as Russian spring-summer encephalitis virus)</p> <p><i>Francisella tularensis</i></p> <p>Hepatitis B virus</p> <p>Herpes B virus</p> <p>Human immunodeficiency virus</p> <p>Highly pathogenic avian influenza virus</p> <p>Japanese encephalitis virus</p> <p><i>Mycobacterium tuberculosis</i></p> <p>Poliovirus</p> <p>Rabies virus</p>

UN # and Proper Shipping Name	Microorganism Classified as Category A in any Form (Always Classified as Category A)	Microorganism Classified as Category A only when Cultured
		<i>Rickettsia prowazekii</i> <i>Rickettsia rickettsii</i> Rift Valley fever virus <i>Shigella dysenteriae</i> type 1 Tick-borne encephalitis virus Venezuelan equine encephalitis virus West Nile virus Yellow fever virus <i>Yersinia pestis</i>
<p style="text-align: center;"><b>UN 2900</b></p> <p style="text-align: center;">Infectious substance, affecting animals</p>		African swine fever virus Avian paramyxovirus Type 1- Velogenic Newcastle disease virus Classical swine fever virus Foot and mouth disease virus Lumpy skin disease virus <i>Mycoplasma mycoides</i> – Contagious bovine pleuropneumonia Peste des petits ruminants virus Rinderpest virus Sheep-pox virus Goatpox virus Swine vesicular disease virus Vesicular stomatitis virus

\* This list is not exhaustive

[Back to XII Shipping Regulation](#)