GUIDELINES FOR HANDLING PATHOGENIC MICROORGANISMS AND DISPOSING BIOHAZARDOUS WASTE

Biohazard Recognition and Control

Institutional Biosafety Committee
University of Wisconsin–Madison
www.fpm.wisc.edu/biosafety
Inside Cover

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Additional copies of this book can be obtained from the Office of Biological Safety

Cover Emblem: Universal Biohazard Symbol
Signifies actual or potential contamination of equipment, rooms, materials, or animals by viable infectious agents.
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Introduction

This booklet seeks to increase awareness of biological hazards commonly encountered in research, clinical, and teaching laboratories at the University of Wisconsin–Madison, and to provide guidance on recommended practices. Biological hazards include microorganisms, animal, plant or human-derived substances, genetically modified biological materials and research animals or their tissues, from which transmission of infectious agents or toxins is reasonably anticipated. Containment practices are also used to protect the environment from exotic, novel and transgenic organisms whose escape from the laboratory must be prevented.

The goal of safety awareness and practice is to assure personnel that—with proper precautions, equipment, and facilities—most biohazardous materials can be handled without undue risk to themselves, their associates, their families, and the environment. The biosafety principles described are based on sound safety practices, common sense, good housekeeping, thorough personal hygiene, regulatory guidance documents and a plan for responding to accidents. Well-organized and procedurally disciplined laboratories are often more effective scientifically, in addition to being safer.

This document is intended not only for trained microbiologists, but also for individuals handling potentially biohazardous materials in other laboratory disciplines such as biochemistry, genetics, oncology, immunology, molecular biology, plant pathology, animal sciences, and biomedical engineering. Persons who have little microbiological training might not realize the potential hazard involved. While this document focuses on hazards that are biological in origin, biological research often involves use of chemicals that are human health hazards, such as carcinogens, teratogens, and drugs. Precautions for handling chemicals are described in the UW Laboratory Safety Guide.

This manual serves as a general biological safety manual for this institution and provides a document that also is useful for training. Laboratories should supplement this manual with additional information that describes the specific hazards and mitigating measures that are used in their facility. Campus investigators contemplating research involving biological hazards and recombinant DNA activities must register their research with the UW-Madison Office of Biological Safety (OBS).
General Principles of Biological Safety

Risk Assessment

Risk assessment is the rational application of safety principles to available options for handling hazardous materials. The following characteristics are considered when evaluating a potential pathogen:

- The agent’s biological and physical nature
- The sources likely to harbor the agent
- Host susceptibility
- The procedures that may disseminate the agent
- The best method to effectively inactivate the agent

Risk Groups

Microorganisms that are human pathogens can be categorized into risk groups (RG) based on the transmissibility, invasiveness, virulence (i.e., ability to cause disease), and the lethality of the specific pathogen. Risk groupings of infectious agents (RG1 through RG4) approximately correspond to biosafety levels (BSL1 through BSL4), which describe containment practices, safety equipment, and facility design features recommended for safe handling of these microorganisms. A parallel series of animal biosafety levels (ABSL1 through ABSL4) applies to handling of infected or potentially infected animals.

Beginning with RG1 agents, which are nonpathogenic for healthy human adults, the scheme ascends in order of increasing hazard to RG4. The risk group listing of the NIH Guidelines for Research Involving Recombinant DNA Molecules (see Appendix A) is an accepted standard, even when recombinant DNA technology is not used. The American Biological Safety Association also provides a comprehensive risk group listing that references agencies globally. The Material Safety Data Sheets (MSDS) for Infectious Substances available through the Health Canada website are an excellent source of information about pathogens.

**RISK GROUP 1** agents are not associated with disease in healthy adult humans. Examples: *E. coli* K-12, *Saccharomyces cerevisiae*.

**RISK GROUP 2** agents are associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available. Examples: enteropathogenic *E. coli* strains, *Salmonella, Cryptosporidium, Staphylococcus aureus*.

**RISK GROUP 3** agents are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk). Examples: human immunodeficiency virus, *Brucella abortus, Mycobacterium tuberculosis*.

**RISK GROUP 4** agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk). Examples: Ebola virus, Cercopithecine herpesvirus 1 (Herpes B or Monkey B virus).
Consideration of the risk group assignment, however, merely is a starting point for the comprehensive risk assessment. Further attention must be given to the circumstances, such as the planned procedures and the available safety equipment. Then, the recommended precautions may be increased or decreased relative to those based solely on the risk group assignment and adjusted to reflect the specific situation in which the pathogen will be used.

Microorganisms in RG1 require use of standard basic biological laboratory facilities and microbiological practices, whereas those in RG4 require maximum containment facilities and practices. Some of the agents likely to be handled experimentally at UW-Madison are RG2 or RG3 pathogens; designated as moderate and high hazard, respectively. These agents typically require more sophisticated engineering controls (e.g., facilities and equipment) than are available in standard laboratories, as well as special handling and decontamination procedures. Consideration also is extended to microorganisms that cause diseases in animals and/or plants, which are not categorized into risk groups as are human pathogens. The desired containment for animal and plant pathogens is based on the severity of the disease and its ability to disseminate and become established in the local environment.

The progression from invasion to infection to disease following contact with an infectious agent depends upon the dose, route of transmission, invasive characteristics of the agent, virulence and resistance of the exposed host. Not all contacts result in infection and even fewer develop into clinical disease. Even when disease occurs, severity can vary considerably. Attenuated strains should be handled with the same precautions as the virulent strain unless the reduced pathogenicity is well documented and is irreversible. Viral vectors, even if rendered replication defective, still may pose a threat of recombination with wild-type strains and/or unintentional delivery of their foreign genes. It is prudent to assume virulence and to handle such agents with precautions appropriate for the virulent parental organism.

**Routes of Infection**

Pathogens are transmitted via several routes of infection, depending on the pathogen in question. The most common routes of infection are inhalation of infectious aerosols or dusts, exposure of mucous membranes to infectious droplets, ingestion from contaminated hands or utensils, or percutaneous self-inoculation (injection or incision). Increased risk is associated with pathogens that are aerosol transmitted and when high concentrations or large volumes are used. Appropriate precautions can be implemented to avoid such exposures.

**Exposure Sources**

**Clinical and Pathological Specimens**

Every specimen from humans or animals may contain infectious agents. Human specimens should be considered especially hazardous. Personnel in laboratories and clinical areas handling human blood or body fluids practice Universal Precautions, an approach to infection control wherein all human blood and certain human body fluids are treated as if known to be infectious for human immunodeficiency virus (HIV), hepatitis B virus (HBV), and other bloodborne pathogens. Such personnel are required by law to complete Bloodborne Pathogen training. The Occupational Health Office administers the UW-Madison Bloodborne Pathogens Program, which provides mandatory training and makes HBV immunization available.
A written exposure control plan must be prepared by laboratories that handle human blood or other potentially infectious materials (PIM), defined in the regulations as semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva in dental procedures, and any body fluids in situations where it is difficult or impossible to differentiate between body fluids. Any unfixed human tissue, organ, or primary cell cultures, and HIV- or HBV-containing culture media or other solutions are also subject to oversight. Blood, organs, or other tissues from experimental animals infected with HIV or HBV are also included. Contact the UW-Madison Occupational Health Officer for more information on precautions and regulatory requirements.

Cultures

Routine manipulations of cultures may also release microorganisms via aerosol formation:

- Popping stoppers from culture vessels
- Opening vessels after vigorous shaking
- Flame-sterilizing utensils, which causes spatter
- Centrifuge
- Sonicator
- Expelling the final drop from a pipette

Manipulate cultures of infectious material carefully to avoid aerosols. Centrifugation should involve the use of gasket-sealable tubes and rotors. Seal microplate lids with tape or replace the lids with adhesive-backed Mylar film. Load, remove, and open tubes, plates, and rotors within a biological safety cabinet (BSC) or fume hood. Accidental spilling of liquid infectious cultures is an obvious hazard due to the generation of aerosols (airborne droplets containing microorganisms).

Equipment used for manipulations of infectious materials, such as sonicators, flow cytometers, cell sorters, and automated harvesting equipment, must be evaluated to determine the need for secondary containment and to consider decontamination issues. When preparing aliquots of infectious material for long-term storage, consider that viable lyophilized cultures may release high concentrations of dispersed particles if ampoules are not properly sealed. Breakage of ampoules in liquid nitrogen freezers may also present hazards because pathogens may survive and disperse in the liquid phase.

Use of human or animal cell cultures in laboratories requires special consideration. Cell or tissue cultures in general present few biohazards, as evidenced by their extensive use and lack of infection transmitted to laboratory personnel. Clearly, when a cell culture is inoculated with or known to contain a pathogen, it should be classified and handled at the same biosafety level as the agent. BSL2 containment conditions are used for cell lines of human origin, even those that are well established, such as HeLa and Hep-2, and for all human clinical material (e.g., tissues and fluids obtained from surgery or autopsy). Cell lines exposed to or transformed by an oncogenic virus, primate cell cultures derived from lymphoid or tumor tissue, and all nonhuman primate tissues are handled using BSL2 practices. A biological safety cabinet, not a laminar flow clean bench, should be used for manipulations that have potential to create aerosols.
**Animals**

Exercise care and thoughtfulness when using animals in research. Numerous risks may be present when animals are used in studies of microorganisms, as well as studies of hazardous chemicals. Use containment and personal protective equipment (PPE) that protects against both the biological and chemical hazards. Precautions commonly include use a lab coat, gloves and eye protection when handling animals and their bedding; respiratory protection may be recommended when specific conditions present a concern.

There are some inherent risks in working with animals (e.g., allergenicity, bites, and scratches). Laboratory and wild-trapped animals may harbor microorganisms that can produce human diseases following bites, scratches, or exposure to excreted microorganisms. Rhesus macaques present a significant potential for hazards, requiring that stringent procedures be followed to guard against Herpes B virus (Cercopithecine herpesvirus 1). Even in the absence of known hazards, animal care providers should use precautions to avoid exposure to animal allergens.

In the process of inoculating animals, an investigator can be exposed to infectious material by accidental self-inoculation or inhalation of infectious aerosols. During surgical procedures, necropsies, and processing of tissues, aerosols can be produced inadvertently, or the operator can inflict self-injury with contaminated instruments. Since animal excreta can also be a source of infectious microorganisms, investigators should take precautions to minimize aerosols and dust when changing bedding and cleaning cages. Containment equipment such as a fume hood or biosafety cabinet is sometimes appropriate for doing cage changes. Bedding from animals infected with pathogens and those potentially infected must be decontaminated prior to disposal, typically by autoclaving.

Transfer of human cells, primate cells or opportunistic microbes; whether newly isolated or well established, into immunocompromised animals could result in propagation of pathogens that would be suppressed in the normal host. BSL2 containment must be applied to mitigate against such risks and also to prevent spread of animal pathogens within a research colony.

Some research animals are treated with hazardous chemicals. Handling of the hazardous chemicals, administration of the chemicals to animals, and handling of these animals, animal tissues (necropsy), and their wastes must be done with appropriate containment and PPE. Preparation of stock solutions of hazardous chemicals (even small amounts of volatile hazardous chemicals), preparation of animal feed containing hazardous chemicals, and cage changes of animals with hazardous chemicals in their wastes are all steps best done in the fume hood. Bedding contaminated chemical or radioactive hazardous substances must be decontaminated prior to disposal; the Chemical Safety and Radiation Safety Offices should be consulted for this determination.

**Plant Bioccontainment**

Biosafety principles are applied to activities involving exotic plants and plant pests, and to transgenic plants and plant pests. Under special circumstances, which typically require explicit approval from USDA-APHIS, it is possible to conduct field trials. Otherwise, release to the environment must be prevented.
Containment may be achieved by a combination of physical and biological means. Containment for transgenic plants and their associated plant pathogens relies more heavily on biological factors than is the norm for human and animal infectious agents. The goal is to protect the environment, not the researcher. The risk assessment considers the specific organism(s), geographic/ecological setting, and available mechanical barriers; the selected practices are tailored to the specific situation. It becomes especially difficult to prescribe containment when genetic modifications lead to uncertainty in characteristics such as host range and competitiveness.

For research involving plants, four biosafety levels (BSL1-P through BSL4-P) are utilized (see Appendix P, NIH Guidelines for Research Involving Recombinant DNA Molecules). BSL1-P is designed to provide a moderate level of containment for experiments for which there is convincing biological evidence that precludes the possibility of survival, transfer, or dissemination of recombinant DNA into the environment, or in which there is no recognizable and predictable risk to the environment in the event of accidental release. BSL2-P is designed to provide a greater level of containment for experiments involving plants and certain associated organisms for which there is a recognized possibility of survival, transmission, or dissemination of recombinant DNA–containing organisms, but the consequence of such an inadvertent release has a predictably minimal biological impact. BSL3-P and BSL4-P describe additional containment conditions for research with plants and certain pathogens and other organisms that require special containment because of their recognized potential for significant detrimental impact on managed or natural ecosystems.

BSL1-P relies upon accepted scientific practices for conducting research in most ordinary greenhouse or growth chamber facilities and incorporates accepted procedures for good pest control and horticultural practices. BSL1-P facilities and procedures provide a modified and protected environment for the propagation of plants and microorganisms associated with the plants and a degree of containment that adequately controls the potential for release of biologically viable plants, plant parts, and microorganisms associated with them. BSL2-P and BSL3-P rely upon accepted scientific practices for conducting research in greenhouses with organisms infecting or infesting plants in a manner that minimizes or prevents inadvertent contamination of plants within or surrounding the greenhouse.

**Laboratory Exposure**

**Teaching Laboratories**

Whenever possible, we recommend the use of avirulent strains of infectious microorganisms in teaching laboratories. However, even attenuated microbes should be handled with care. Students should be cautioned against and trained to prevent unnecessary exposure, as exposure to “avirulent” strains may be problematic in the immunocompromised individual. Establishment of safety consciousness and safe practices are essential to the conduct of good science.

**Research Laboratories**

Experiments in research laboratories using high concentrations or large quantities of pathogens increase the risk of exposure and the possibility of overcoming natural barriers to infection. The use of animals in research on infectious diseases also presents greater opportunities for exposure.
Clinical Laboratories

Personnel in laboratories performing diagnostic tests of clinical specimens from human or animal patients are often at risk of exposure to infectious agents. The absence of an infectious disease diagnosis does not preclude the presence of pathogens. This is especially true of materials from patients who receive immunosuppressive therapy, since such treatment may activate latent infectious agents.

Health Status

Some unusual circumstances warrant special considerations or measures to prevent infection of laboratory personnel by certain microorganisms. Certain medical conditions increase your risk of potential health problems when working with pathogenic microorganisms and/or animals. These conditions can include pregnancy, immunosuppression, and animal-related allergies. If any of these conditions apply to you, inform your personal physician/health care professional of your work.

Table 1. Relationship of Risk Groups to Biosafety Levels, Practices, Facilities, and Equipment

<table>
<thead>
<tr>
<th>Risk Group (RG)</th>
<th>Biosafety Level (BSL)</th>
<th>Examples of Laboratories</th>
<th>Laboratory Practices</th>
<th>Facilities and Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>RG 1</td>
<td>Basic BSL 1</td>
<td>Basic teaching and research</td>
<td>Good microbiological technique (GMT)</td>
<td>None required; open bench work, directional airflow(^a)</td>
</tr>
<tr>
<td>RG 2</td>
<td>Basic BSL 2</td>
<td>Primary health services; research; diagnostic, teaching and public health</td>
<td>BSL 1 practices plus protective clothing; biohazard sign</td>
<td>Open bench plus biological safety cabinet (BSC) for potential aerosols</td>
</tr>
<tr>
<td>RG 3</td>
<td>Containment BSL 3</td>
<td>Special diagnostic and research</td>
<td>BSL 2 practices plus special clothing, controlled access, directional airflow</td>
<td>BSC and/or other primary containment devices for all activities</td>
</tr>
<tr>
<td>RG 4</td>
<td>Maximum Containment BSL 4</td>
<td>Dangerous pathogen unit</td>
<td>BSL 3 practices plus airlock entry, shower exit, special waste disposal</td>
<td>Class III BSC or positive pressure suits, double-door autoclave, filtered air</td>
</tr>
</tbody>
</table>

\(^a\) OSHA Laboratory Standard, (29 CFR)


Biohazard Containment

Although the most important aspect of biohazard control is the awareness and care used by personnel in handling infectious materials, certain features of laboratory design, ventilation, and safety equipment can prevent dissemination of pathogens and exposure of personnel should an accidental release occur.

Practices and Procedures

The following practices are important not only for preventing laboratory infection and disease, but also for reducing contamination of experimental material.

Standard microbiological practices are common to all laboratories. Special microbiological practices enhance worker safety, environmental protection and address the risk of handling agents requiring increasing levels of containment. Please also see the DCE Biosafety in Microbiological and Biomedical Laboratories (BMBL) website at http://www.cdc.gov/biosafety/publications/index.htm (Section IV) regarding information on Laboratory Biosafety Level criteria, etc.

These standardized practices and procedures provide the foundation for the more restrictive containment of RG3 organisms, which are not covered in this manual. Specialized facilities and rigorous attention to procedures that control the biohazards are required for the conduct of research under BSL3 containment, which must be described in a biosafety manual that is specific to the agents, facilities, and activities. Points to consider in writing the BSL3 manual are described in Appendix B.

Good Microbiological Technique and Personal Hygiene: Biosafety Level 1

✓ Do not eat, drink, chew gum, use tobacco, apply cosmetics, or handle contact lenses in the work area.
✓ Do not store food for human consumption in the work area.
✓ Do not store items such as coats, handbags, dishes or other personal items in the laboratory.
✓ Wash hands frequently after handling infectious materials, after removing gloves and protective clothing, and always before leaving the laboratory.
✓ Keep hands away from mouth, nose, eyes, face, and hair.
✓ Use mechanical pipetting devices; never mouth-pipette.
✓ Wear appropriate Personal Protective Equipment. A lab coat and eye protection are the minimum, with gloves and respiratory protection added to suit the activities.

Laboratory Procedures for Handling Infectious Microorganisms: Biosafety Level 2

✓ Prepare a site-specific laboratory safety manual outlining activities and defining standard operating procedures.
✓ Train employees and ensure that all personnel are informed of hazards.
✓ Plan and organize materials/equipment before starting work.
✓ Keep laboratory doors closed; limit access to personnel who have a need to be in the lab.
✓ Post a biohazard sign at the laboratory entrance when RG2 pathogens are used. Identify the agents in use and the appropriate emergency contact personnel. Biohazard signs and laboratory information signs are available from the Office of Biological Safety.

✓ Wear a fully fastened laboratory coat, gloves, and eye protection when working with infectious agents or potentially hazardous materials, including human blood, body fluids, tissue and cells.

✓ Remove all protective clothing, including gloves, and leave within the laboratory before exiting.

✓ When practical, perform all aerosol-producing procedures such as shaking flasks, grinding tissue, sonicating, mixing, and blending in a certified biological safety cabinet. Note that some equipment may compromise cabinet function by disturbing the air curtain.

✓ Centrifuge materials containing infectious agents in unbreakable, closable tubes. Use a rotor with a sealed head or safety cups, and load it in a biological safety cabinet. After centrifugation, open the rotor and tubes in a biological safety cabinet.

✓ Avoid using hypodermic needles whenever possible. If it is necessary to use them, discard used syringe-needle units in a sharps container without removing or re-capping the needles.

✓ Cover counter tops where hazardous materials are used with plastic-backed disposable paper to absorb spills; discard it at the end of the work session.

✓ Routinely wipe work surfaces with an appropriate disinfectant after experiments and immediately after spills.

✓ Routinely decontaminate all infected materials by appropriate methods before disposal.

✓ Report all accidents and spills to the laboratory supervisor. All laboratory personnel should be familiar with the emergency spill protocol, the location of cleanup equipment and the First Report of Exposure/Release Form (available on the OBS website).

✓ Good housekeeping practices are essential in laboratories engaged in work with infectious microorganisms. Establish the habit of weekly cleaning.

✓ Be sure to advise custodial staff of hazardous areas and places they are not to enter. Use appropriate warning signs.

**Personal Protective Equipment**

Laboratory coats provide a barrier that protects the worker from hazardous materials contacted in the laboratory. Note that it is not possible to see residues of many hazardous materials; they could have been left behind on various surfaces by another worker. By removing your lab coat when exiting the lab, contaminants remain in the lab. It follows logically then that protective clothing should not be taken home for cleaning. Depending on the nature of the work, protective clothing also could include disposable sleeves, coats that close in back, disposable protective suits (e.g. Tyvek) and hair and shoe covers.

Gloves should be worn whenever there is the potential for contact with hazardous materials. They further serve to maintain the integrity of the material being handled. Many different types of gloves are available, and the choice depends on the nature of the hazard. Gloves must be removed before exiting the lab. Material that is transported outside the lab that poses a risk to personnel should be surface decontaminated and placed in a clean secondary container so that gloves need not be worn outside the lab.
The eyes and mucous membranes are vulnerable routes of exposure. Eye protection should always be worn in the laboratory. Contact lenses may be worn with discretion and in combination with eye protection. Depending on the activities, it may be appropriate to use safety glasses with side shields, goggles, and/or a splash shield. The Environment, Health & Safety Department (EHS) offers prescription safety glasses at minimal or no cost to help employees comply with the OSHA Lab Standard.

Respiratory protection should be considered carefully and used only when there is risk of aerosol exposure that cannot be mitigated through the use of alternative procedures or containment equipment. The background level of microbes in the research laboratory should be negligible when good microbiological techniques are employed. Selection of a respirator to guard against pathogens is not as simple as for chemical hazards where tables of permissible exposure limits are available and background levels are factored into the decision. Recommendations for respirators are not documented for work with pathogens with the exception of clinical specimens containing *Mycobacterium tuberculosis* since acceptable exposure levels have not been determined.

An issue regarding respiratory protection is that, if used improperly, the user has a false sense of security. A surgical mask or common dust mask, have poor fit to the contours of the face, provide minimal protection against large particles and are inappropriate for work with infectious agents. A HEPA (high efficiency particulate air) filtered face piece (e.g., N95 or N100) is appropriate for many situations where protection against animal allergens and microbes is desired, but the protection will only be as good as the respirator’s fit to the face. Furthermore, HEPA filtration is ineffective against volatile chemicals. A full head cover with a Powered Air Purifying Respirator (PAPR) is used when respiratory protection is critical for work with highly pathogenic microbes or in situations where a biological safety cabinet cannot be used. A medical evaluation to wear a respirator, fit testing, and training in proper use are mandatory if respiratory protection is required by the employer. Contact the Occupational Health Office for guidance on appropriate respiratory protection.

**Engineering Controls**

Table 2 describes the relationship between biosafety levels and engineering controls, which include lab design, lab ventilation, and biological safety cabinets.

**Laboratory Design**

The more virulent an organism, the greater the degree of physical containment required. Proper safety equipment provides primary containment; laboratory design provides secondary containment. The Office of Biological Safety is available for consultation on these matters.

**Laboratory Ventilation**

For containment in a laboratory to be effective, it is important that laboratory air pressure be lower than that in the adjacent spaces. This negative air pressure differential ensures that air will enter the laboratory and not egress to the hallway or adjacent rooms. **To maintain negative room pressure, laboratory doors must be kept closed.** Exhaust air from biohazardous laboratories should not be recirculated in the building. It should be ducted to the outside and released from a stack remote from the building air intake. In certain special situations, air exhausting from a hazardous facility should be filtered through certified HEPA (high efficiency particulate air) filters that are tested at least annually and verified to retain microorganisms.
Table 2. Summary of Facility Standards Recommended for Biosafety Levels

<table>
<thead>
<tr>
<th>Facility Standard</th>
<th>BSL 1</th>
<th>BSL 2</th>
<th>BSL 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory visit by Office of Biological Safety</td>
<td>Desirable</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Isolation of laboratory from public areas</td>
<td>---</td>
<td>---</td>
<td>Desirable</td>
</tr>
<tr>
<td>Eyewash, plumbed</td>
<td>Desirable</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Interior surfaces (impervious, cleanable):</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Bench tops</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Laboratory furniture</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Floors, conventional (no carpet)</td>
<td>Yes</td>
<td>Yes</td>
<td>---</td>
</tr>
<tr>
<td>Floors, seamless, integral cove base</td>
<td>---</td>
<td>Desirable</td>
<td>Yes</td>
</tr>
<tr>
<td>Ceiling, conventional</td>
<td>Yes</td>
<td>Yes</td>
<td>---</td>
</tr>
<tr>
<td>Ceiling, permanent</td>
<td>---</td>
<td>---</td>
<td>Yes</td>
</tr>
<tr>
<td>Sinks in laboratory</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Hands-free</td>
<td>---</td>
<td>---</td>
<td>Yes</td>
</tr>
<tr>
<td>Water supply protected</td>
<td>---</td>
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<td>Yesa</td>
</tr>
<tr>
<td>Windows allowed</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>May be opened</td>
<td>Noa</td>
<td>Noa</td>
<td>No</td>
</tr>
<tr>
<td>Must be sealed</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Room penetrations sealed for gas decontamination (pressure decay testing)</td>
<td>No</td>
<td>No</td>
<td>Desirable</td>
</tr>
<tr>
<td>Ventilation (single-pass supply/exhaust)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Inward air flow (negative pressure)</td>
<td>Yesa</td>
<td>Yesa</td>
<td>Yes</td>
</tr>
<tr>
<td>Mechanical, centralized system</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Mechanical, independent system</td>
<td>No</td>
<td>No</td>
<td>Desirable</td>
</tr>
<tr>
<td>Filtered exhaust required</td>
<td>No</td>
<td>No</td>
<td>Desirable</td>
</tr>
<tr>
<td>Interlocked supply required</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>Annually test filters/HVAC systems</td>
<td>No</td>
<td>No</td>
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</tr>
<tr>
<td>Annually test controls/alarms</td>
<td>No</td>
<td>No</td>
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</tr>
<tr>
<td>Doors (self-closing):</td>
<td>Desirable</td>
<td>Desirable</td>
<td>Yes</td>
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<td>Double-door entry required</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Airlock with shower required</td>
<td>No</td>
<td>No</td>
<td>Desirable</td>
</tr>
<tr>
<td>Autoclave on site</td>
<td>Desirable</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>In laboratory room</td>
<td>---</td>
<td>---</td>
<td>Desirable</td>
</tr>
<tr>
<td>Pass-through (double-ended)</td>
<td>---</td>
<td>---</td>
<td>Desirable</td>
</tr>
<tr>
<td>Biological safety cabinets</td>
<td>Annual certification</td>
<td>Desirable</td>
<td>Yes</td>
</tr>
<tr>
<td>Class I or Class II</td>
<td>---</td>
<td>Desirable</td>
<td>Yes</td>
</tr>
<tr>
<td>Class III</td>
<td>---</td>
<td>---</td>
<td>Desirable</td>
</tr>
<tr>
<td>Vacuum lines should be protected with liquid trap &amp; in-line HEPA filter</td>
<td>Desirable</td>
<td>Yes</td>
<td>Yesb</td>
</tr>
<tr>
<td>Waste effluent treatment</td>
<td>---</td>
<td>---</td>
<td>Desirable</td>
</tr>
<tr>
<td>Centrifuge with sealed rotors</td>
<td>---</td>
<td>Desirable</td>
<td>Yes</td>
</tr>
</tbody>
</table>

--- not applicable or needed

a Required by current Wisconsin Administrative Code [https://docs.legis.wisconsin.gov](https://docs.legis.wisconsin.gov)
b HEPA filter required

Existing facilities that do not meet these recommendations may need to address deficiencies during future maintenance or remodeling. Contact the Office of Biological Safety for assistance.
Types of Ventilation Equipment

Be sure you know the differences between chemical fume hoods, clean benches, biological safety cabinets, and isolators. These provide three basic types of protection:

- **Personal protection** is the protection of the people working in the lab.
- **Product protection** is the protection of the product or experiment.
- **Environmental protection** is the protection of the environment outside the lab.

Different types of ventilation equipment provide different types of protection (see Table 3).

**Chemical Fume Hoods**

**Characteristics**
- Offer only protection of personnel.
- Always exhaust air to the outside.
- Do not offer protection to the product or the environment, as there is no filtration of intake and exhaust air; sometimes air cleaning treatment is added to the exhaust.
- Air from the laboratory is directly drawn over the product in the hood.

**Applications**
- Used for work with chemical hazards; also used to prevent laboratory exposure to biological materials when product protection (sterility) is not a concern.

**Clean Benches, Clean Air Devices**

**Characteristics**
- Provide product protection only.
- Product protection is provided by creating a unidirectional airflow generated through a HEPA filter.
- Discharge air goes across the work surface and directly into workroom.

**Applications**
- Any application where the product is not hazardous but must be kept contaminant free.
- Preparation of nonhazardous mixtures and media.
- Particulate-free assembly of sterile equipment and electronic devices.

**Biological Safety Cabinet (BSC)**

**Characteristics**
- Designed to contain biological hazards and to allow products to be handled in a clean environment.
- Inward airflow for personal protection.
- HEPA-filtered exhaust air for environmental protection.
- HEPA-filtered supply air for product protection (except Class I).
- Separated into classes and types: Class I, Class II (Type A1/A2/B1/B2), Class III (glove box, isolator).

**Applications**
- Microbiological studies.
Cell culture research and procedures.
Protection against hazardous chemicals varies according to the class and type.
Pharmaceutical research, manufacturing, and quality control testing.

**Biological Safety Cabinets**

Biological safety cabinets (BSCs) are the primary means of containment developed for working safely with infectious microorganisms. When certified and used correctly in conjunction with good microbiological techniques, they can control infectious aerosols. BSCs are designed to provide personal, environmental, and product protection when appropriate practices and procedures are followed. An excellent reference is *Primary Containment for Biobezards: Selection, Installation, and Use of Biological Safety Cabinets*, published by the CDC and NIH. See also the UW OBS website under the Facility and Containment tab.

Laminar flow clean benches are not biological safety cabinets and should never be used for work with potentially hazardous biological or chemical materials. These devices protect the material in the cabinet but not the worker or the environment.

**BSC Types**

Three kinds of biological safety cabinets, designated as Class I, II, and III, have been developed to meet varying research and clinical needs. Table 3 summarizes the major characteristics of the various types. Four varieties of Class II biological safety cabinets are used on campus. All are adequate for manipulations of pathogens in RG2 or RG3.

Please note that because of the greater safety margin, small amounts of volatile chemical toxins or radioactive materials can be used in Type B cabinets. Type A cabinets, however, recirculate a high percentage of air and therefore cannot be used with toxic, explosive, flammable, or radioactive substances. Class III cabinets and isolators are totally enclosed glove boxes, which are used for the most hazardous biological operations and for super-clean manufacturing. These enclosures should not be confused with anaerobic chambers.

The ultraviolet lamps within some biosafety cabinets provide only limited ability to inactivate microbes. The light is effective only on surfaces it contacts. UV light has little ability to penetrate organic material such as thin films of oil. The UV output of the lamp decreases as the lamp ages and decreases further unless the lamp is cleaned periodically to remove dust and oil films. Furthermore, exposure to UV light may cause eye injury and/or erythema (sunburn). Our recommendation is to thoroughly disinfect surfaces and equipment with an appropriate disinfectant instead of relying on UV light.

**Purchasing a BSC**

A contract is maintained by the UW-Madison for the purchase of all types of biological safety cabinets and laminar flow benches. Before ordering one, consult the Office of Biological Safety or the Environmental Health Program for an evaluation of BSC suitability for the intended work and of the available space. To ensure the adequacy of the installed mechanical ventilation and to facilitate coordination with the Physical Plant Remodeling group, exhausted biological safety cabinets (type A or B) must be approved by the Engineering Department, UW Facilities Planning & Management, prior to purchase.
## Table 3. Ventilation Equipment

<table>
<thead>
<tr>
<th>Device</th>
<th>Protection</th>
<th>Airflow Direction (feet/min)</th>
<th>Application/Airflow Pattern</th>
<th>Use of Volatile Toxic Chemicals and Radionuclides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical fume hood</td>
<td>Personnel only</td>
<td>Inward (100)</td>
<td>A completely exhausted, unfiltered device used for work with chemical hazards, minimizing exposure to personnel.</td>
<td>Acceptable</td>
</tr>
<tr>
<td>Clean air device, Clean bench</td>
<td>Product only</td>
<td>Outward (100)</td>
<td>Any application where the product is not hazardous, but must be kept contaminant free. A laminar flow clean bench provides HEPA filtered supply to the work surface and a particulate-free work area. Preparation of nonhazardous intravenous mixtures and media. Particulate-free assembly of sterile equipment and electronic devices. Polymerase chain reaction (PCR).</td>
<td>Not Acceptable</td>
</tr>
<tr>
<td>Animal transfer station</td>
<td>Product and 70% personnel</td>
<td>Inward</td>
<td>A HEPA-filtered device used to transfer animals from dirty to clean cage, minimizing exposure to animal and personnel.</td>
<td>Not Acceptable</td>
</tr>
<tr>
<td>Bedding dump station</td>
<td>Personnel and environment</td>
<td>Inward</td>
<td>A HEPA-filtered device used to capture airborne particulates when disposing of waste bedding from animals, minimizing exposure to personnel.</td>
<td>Not Acceptable</td>
</tr>
<tr>
<td>BSC Class I</td>
<td>Personnel and environment</td>
<td>Inward</td>
<td>Effectively a fume hood with filtered exhaust. HEPA filtered exhaust air passes through a dedicated duct system to the outside</td>
<td>Acceptable if connected to exhaust(^1)</td>
</tr>
<tr>
<td>BSC Class II–A1</td>
<td>Product, personnel, and environment</td>
<td>Inward (75)</td>
<td>A laminar flow device that recirculates 70% of its airflow to the work surface through a HEPA filter and exhausts the 30% balance through a HEPA filter back into the room or to the outside through a thimble connection via building exhaust system. Plenums are under <strong>positive</strong> pressure.</td>
<td>Minute amounts only if thimble connected to exhaust(^1)</td>
</tr>
<tr>
<td>BSC Class II–A2</td>
<td>Product, personnel, and environment</td>
<td>Inward (100)</td>
<td>A laminar flow device that recirculates 70% of its airflow to the work surface through a HEPA filter and exhausts the 30% balance through a HEPA filter back into the room or to the outside through a thimble connection via building exhaust system. Plenums are under <strong>negative</strong> pressure.</td>
<td>Minute amounts only if thimble connected to exhaust(^1)</td>
</tr>
<tr>
<td>BSC Class II–B1</td>
<td>Product, personnel, and environment</td>
<td>Inward (100)</td>
<td>A laminar flow device that recirculates 30-40% of its airflow to the work surface through a HEPA filter and exhausts the 60-70% balance through a HEPA filter to the outside via building exhaust system. Exhaust connection must be hard ducted to the outside.</td>
<td>Limited amounts(^1)</td>
</tr>
<tr>
<td>BSC Class II–B2</td>
<td>Product, personnel, and environment</td>
<td>Inward (100)</td>
<td>A laminar flow device that has a dedicated HEPA filtered supply to the work surface and a dedicated HEPA filtered exhaust to the outside via building exhaust system. No recirculated supply, and exhaust connection must be hard ducted to the outside.</td>
<td>Acceptable</td>
</tr>
<tr>
<td>BSC Class III, Isolator, Glove box</td>
<td>Maximum product, personnel, and environment</td>
<td>Inward</td>
<td>A laminar flow device with dedicated HEPA filtered supply to the work surface and dual dedicated HEPA filtered exhaust to the outside via building exhaust system. No recirculated supply, and exhaust connection must be hard ducted to the outside. (e.g., pharmaceutical quality control testing, super-clean manufacturing without creating clean room, pharmaceutical manufacturing of potent compounds, BL4 agents).</td>
<td>Limited amounts(^1)</td>
</tr>
</tbody>
</table>

\(^1\) In no circumstances should the chemical concentration approach the lower explosion limits of the compound.
Proper Use of a BSC

Loading Materials/Equipment and BSC Startup

- Always close doors to lab when working with any biohazardous materials.
- Turn on blower at least 10 minutes before use and make sure drain valve is closed.
- Check pressure gauge(s) to ensure proper operating conditions are within range of those indicated on the annual certification label on the BSC.
- Check grilles for obstructions.
- Disinfect all interior work surfaces with a disinfectant appropriate for the agent in use.
- Disinfect the exterior of all containers prior to placing them in the cabinet.
- Load only items needed for the procedure.
- Arrange materials so that movement within the cabinet is minimized; flow of procedure is from clean to dirty. Never place non-sterile items upstream of sterile items. Check that rear and front grilles are unobstructed. Never hang articles from the interior ceiling grid.
- Once the cabinet is loaded, adjust the view screen to proper position and wait 4 minutes before commencing procedures. Never use the view screen above the mark specified by the certification agency (common opening is 8-inches and up to 12” for animal facilities).
- Restrict traffic in the vicinity of the BSC.

Recommended Work Techniques

- Wash hands thoroughly with soap before and after procedures.
- Wear sterile gloves and lab coat/gown and eye protection; use aseptic technique.
- Avoid blocking front grille. Work only on or over a solid surface and adjust the chair so your armpits are at the level of the lower window edge.
- Avoid rapid movement during procedures, particularly within the BSC, but also in the vicinity of the BSC.
- Move hands and arms straight into and out of the work area; never rotate hand/arm out of work area during procedure. Move laterally in work area.
- Do not use a Bunsen burner that burns gas continuously since the flame causes air turbulence and could cause a fire or explosion. Consider using alternative equipment, such as flameless instrument sterilizers or heat plates.
- Place contaminated items such as pipettes in a waste receptacle located within the BSC.

Final Purging and Wipe-down

- After completing work, run the BSC blower for at least 10 minutes before unloading materials from the cabinet.
- Disinfect the exterior of all containers before removing them from the work zone.
- Decontaminate interior work surfaces of the BSC with an appropriate disinfectant effective against the agent used.
- Routinely check the drip pan beneath the work surface for cleanliness, and if a spill has occurred, clean and disinfect it.
✓ Take care to prevent towelettes from being sucked into exhaust plenums.

**Decontamination and Spills**

All containers and equipment should be surface decontaminated and removed from the cabinet when work is completed. The final surface decontamination of the cabinet should include a wipe-down of the work zone. Investigators should remove their gloves and gowns and wash their hands as the final step in safe microbiological practices.

Small spills within the cabinet can be handled immediately by placing the contaminated absorbent paper toweling into the biohazard waste container. Any splatter onto items within the cabinet, as well as the cabinet interior, should be immediately wiped with a towel dampened with decontaminating solution. Gloves should be changed after the work surface is decontaminated and before clean absorbent toweling is placed in the cabinet. Hands should be washed whenever gloves are changed or removed.

Spills large enough to result in liquids flowing through the front or rear grilles require more extensive decontamination. All items within the cabinet should be surface decontaminated and removed. Beneath the BSC work surface is a drip pan to collect large spills. After ensuring that the drain valve is closed, decontaminating solution can be poured onto the work surface, grilles, and the drain pan. Twenty to thirty minutes is generally considered an appropriate contact time for decontamination, but this varies with the disinfectant and the microbiological agent. The drain pan should be emptied into a collection vessel containing disinfectant. If the drain pan is accessible, wipe it down to remove remaining debris. Should the spilled liquid contain radioactive material, radiation safety personnel should be contacted for specific instructions on conducting a similar procedure.

**Maintenance**

To function adequately, the cabinet airflow must be closely regulated and the HEPA filters must be certified. All biological safety cabinets should be certified annually. **Annual certification is required for work at BSL2 and BSL3.** Certification services are available for a fee through the University Health Services (UHS) Environmental Health Program. For more information about this service, contact the UW-Madison UHS. The EH&S department and website also have references to UHS services.

BSCs must be decontaminated prior to being moved from one space to another. Gas decontamination may be required in some situations when a BSC needs to be disassembled, dismantled, or disposed. Gas decontamination must be done by trained personnel; the UHS Environmental Health Program can assist you.

It is the responsibility of all laboratory staff to effectively decontaminate equipment before it is removed from the lab for maintenance, relocation, sale, or disposal.
Disposal of Wastes from Biological Laboratories

The following biohazardous waste disposal guidelines are designed to protect not only the public and the environment, but also laboratory and custodial personnel, waste haulers, and landfill/incinerator operators at each stage of the waste-handling process. Workers who generate biohazardous waste in the laboratory must assure that the labeling, packaging, and intermediate disposal of waste conform to these guidelines. The appropriate packaging of all waste is fundamental for assuring protection of the handler and proper disposal. A display poster that summarizes sharps and glass disposal is available at the EH&S website and upon request. For additional information see our website Disposal Services information page http://www.ehs.wisc.edu/disposalservices.htm and the UW Laboratory Safety Guide.

Decontamination means a process of reducing the number of disease-producing microorganisms and rendering an object safe for handling.

Disinfection means a process that kills or destroys most disease-producing microorganisms, except spores.

Sterilization means a process by which all forms of microbial life, including spores, viruses, and fungi, are destroyed.

What Is Infectious Waste?

The following items usually are considered to be infectious waste:

- Microbiological laboratory wastes such as cultures derived from clinical specimens and pathogenic microorganisms, and laboratory equipment that has come into contact with them
- Tissues, liquid blood, cells and body fluids from humans
- Tissues, liquid blood, cells and body fluids from an animal that is carrying an infectious agent that can be transmitted to humans
- Contaminated sharps

Other categories of waste that require decontamination before disposal are regulated materials such as recombinant organisms, exotic or virulent plant and animal pathogens and bedding/waste from animals housed under ABSL2 or higher containment. For mixed waste, the hazardous chemical and radioactive materials take precedence over the biological hazards, and special handling may be required.

Infectious and Medical Waste

Contaminated materials from laboratories and animal facilities, such as cultures, tissues, media, plastics, glassware, instruments, and laboratory coats, must be decontaminated before disposal or washing for re-use. Collect contaminated materials in leak-proof containers labeled with the universal biohazard symbol; autoclavable biohazard bags are recommended. After autoclaving, deface the biohazard symbols on containers. Additionally, you should add a green “OK to Trash” sticker to the bags to show the material is decontaminated and safe to handle by UW custodial/waste disposal personnel.

Effective 07/2011. UW’s waste contractor will NOT accept any red biohazard waste bag or container because they consider these waste items to be Medical Waste which has NOT been Autoclaved and is therefore still biohazardous. This is in effect regardless of whether a red bag
has been autoclaved and/or biohazard symbol defaced or covered. Furthermore, the contractor will not accept the UW “OK to Trash” sticker as proof of non-hazardous status on a red bag.

All units that generate biohazard waste must put autoclaved waste in EITHER CLEAR OR ORANGE biohazard autoclave bags. Both clear and orange colored biohazard bags are available via MDS.

Laboratories shall continue to use a green “OK To Trash” sticker on autoclaved waste in clear or orange biohazard bags. The green sticker informs Physical Plant custodial personnel that the bag only contains autoclaved material and does NOT contain SHARPS. Custodians will no longer handle red biohazard bags, even if labeled with a green “OK to Trash” sticker.

Sharps are instruments designed to cut or penetrate skin. Examples include syringes with needles, lancets, and razor blades, regardless of their actual use. Collect these items in rigid puncture-proof containers (preferably a purchased Sharps Container) to prevent wounding of coworkers, and waste handlers. Sharps require special handling and may not go directly to the landfill. A contractor, MERI (Madison Energy Recovery, Inc.), collects and processes medical sharps, disinfecting and grinding them prior to final disposal. Medical sharps need not be autoclaved prior to disposal by MERI unless generated by a BSL3 or Select Agent facility. If your facility is off the main UW-Madison campus, be sure to verify disposition procedures for your sharps and infectious waste as they may differ from buildings on the main campus; building managers may be your best resource. If you plan to autoclave the sharps container, make sure it is made from heat resistant material. Please note that general building custodial personnel are instructed not to handle or transport sharps containers as part of their safety training.

Be aware that there are buildings on campus (such as UW Hospital and Clinics) which may have different waste pick-up policies than those stated here for Physical Plant custodial personnel. Be aware of your building policies and contact the facilities manager in your building.

Noninfectious Waste

The following are usually not included in the definition of infectious waste, but should be placed in containers such as plastic bags prior to disposal to contain the waste. If these items have been mixed with infectious wastes, they have to be managed as though they are infectious.

- Items soiled or spotted, but not saturated, with human blood or body fluids. Examples: blood-spotted gloves, gowns, dressings, and surgical drapes.
- Containers, packages, non-fragile waste glass, laboratory equipment, and other materials that have had no contact with blood, body fluids, clinical cultures, or infectious agents.
- Noninfectious animal waste such as manure and bedding, and tissue, blood, body fluids, or cultures from an animal that is not known or suspected to be carrying an infectious agent transmissible to humans.

As a general rule, materials that can cut, but are not intended to do so, should be disposed in a manner that prevents harm. Examples of such materials include fragile glass, glass slides and cover slips, and pipettes and pipette tips. If a bag is apt to be punctured because of sharp-edged contents, double bagging and boxing may be necessary. Furthermore, the material must be decontaminated prior to disposal if it harbors infectious agents or recombinant materials.

Waste from Animal Experiments
Animal carcasses should be placed in a plastic bag within a cardboard box and frozen for collection and disposal by the Safety Department waste management. Bedding from animals housed under ABSL2 or higher containment must be autoclaved prior to disposal as trash rather than packaged with the carcass. Special arrangements can be made to dispose of certain wastes via caustic digester, which is a preferred method for treating prion-infected animals. The *UW Laboratory Safety Guide* provides detailed information about the process to be used for disposal of materials from animal experiments.

**Methods of Decontamination**

Choosing the right method to eliminate or inactivate a biohazard is not always simple; it is difficult to prescribe methods that meet every contingency. Decisions are best left to the personnel directly involved, provided they are well informed and prepared to verify the effectiveness of the treatment. The choice depends largely on the treatment equipment available, the target organism, and the presence of interfering substances (e.g., high organic content) that may protect the organism from decontamination. Other common factors that influence the efficacy of disinfection are contact time, temperature, water hardness, and relative humidity.

Various treatment techniques are available, but practicality and effectiveness govern which is most appropriate. For example, there is a practical limit to the time that can be spent autoclaving waste, and alternative methods might be more effective and economical. The efficacy of the selected method against the particular biohazard must be documented by reference to accepted procedures or quantitative testing.

Use extreme caution when treating waste that is co-contaminated with volatile, toxic, or carcinogenic chemicals, radioisotopes, or explosive substances. Autoclaving this type of waste may release dangerous gases (e.g., chlorine from bleach) into the air. Such waste should be chemically decontaminated or picked up by the Safety Department for special disposal.

Ideally, biohazardous waste should be decontaminated before the end of each working day unless it is to be picked up for special waste treatment. Biohazardous waste should never be compacted. Ordinary lab wastes should be disposed of routinely as much as possible to reduce the amount requiring special handling.

**Steam Sterilization**

Decontamination is best accomplished by steam sterilization in a properly functioning autoclave that is routinely monitored with a biological indicator such as spores of *Bacillus stearothermophilus*. Indicator tape provides assurance only that a high temperature was reached; it does not indicate it was heated for the proper time. The tops of autoclavable biohazard bags should be opened to allow steam entry. For dry materials, it may be necessary to add water to the package prior to autoclaving.

Although we recommend autoclaving all biohazardous wastes for at least 1 hour, the nature of the waste in a load should determine cycle duration. For example, if the waste contains a dense organic substrate such as animal bedding or manure, 1 hour may be insufficient to inactivate certain pathogens buried within. A considerably longer exposure time, for example, 8 to 12 hours, may be required to effectively decontaminate such waste.

General autoclave safety and use guidelines are available at the OBS website. Additionally you may self-register for an online safety training module *Biosafety 106: Autoclave Use* available on Learn@UW.
Sewage Treatment

Fluid that is contaminated with infectious agents or biological toxins must be rendered safe by chemical or autoclave treatment before sewer disposal. Most fluid waste, including human blood, can be discarded by pouring into the sanitary sewer, followed by flushing with disinfectant and water. Care must be taken to avoid splashing and generating aerosols. The routine processing of municipal sewage provides chemical decontamination. Sewer lines should be decontaminated by flushing with hypochlorite (10% bleach) prior to servicing.

Chemical Disinfection

Where autoclaving is not appropriate or feasible, an accepted alternative is to treat material with a chemical disinfectant, freshly prepared at a concentration known to be effective against the microorganisms in use. The disinfectant of choice should be one that quickly and effectively kills the target pathogen at the lowest concentration and with minimal risk to the user. Allow sufficient exposure time to ensure complete inactivation. Other considerations such as economy and shelf life are also important. The susceptibility to chemical disinfection generally is greater for enveloped viruses than for non-lipid viruses, and greater for vegetative bacteria and fungi than for spores. Mycobacteria are more resistant to inactivation than most bacteria, while prions are notably resistant to most chemicals.

The following brief overview cannot do justice to the complexity of this subject. Additional references should be consulted and testing done to verify the efficacy for the given usage.

Alcohol (ethanol, isopropanol) is effective against vegetative forms of bacteria, including mycobacteria and fungi, and hydrophobic (enveloped) viruses, but will not destroy spores or hydrophilic viruses. The recommended strength is 70–90%; higher levels actually may be less efficacious. Alcohol typically is used for disinfection of instruments or surfaces that have low organic burden. Characteristics limiting its usefulness are flammability, poor penetration of protein-rich materials, and rapid evaporation making extended contact time difficult to achieve. Alcohol-based hand-rubs may be used for the decontamination of lightly soiled hands in situations where proper hand-washing is inconvenient or impossible.

Aldehydes (formaldehyde, glutaraldehyde) have broad germicidal activity, but toxicity to humans limits their usefulness as laboratory disinfectants. Example products: Cidex, Wavicide-01.

Peroxygen compounds provide a wide range of bactericidal, viricidal, and fungicidal activity, although activity is variable against bacterial spores and mycobacteria. Corrosivity varies with different products but is less problematic than with hypochlorite disinfectants. Their good detergent properties combine cleaning with disinfection. Example product: Virkon.

Ethylene oxide sterilizers can provide effective treatment of heat sensitive equipment. Ethylene oxide is a human carcinogen. Release of ethylene oxide gas is restricted under federal and state regulations. You must consult with the EH&S Department prior to purchasing this equipment.

Halogens such as hypochlorite, the active ingredient in household bleach, are inexpensive and are also highly effective in decontaminating large spills. Their drawbacks include short shelf life, easy binding to nontarget organic substances, and corrosiveness, even when diluted. Household bleach typically contains 5.25% NaOCl and is diluted 1:10 to 1:100 such that the available halogen (hypochlorite) is 0.05 to 0.5%. Solutions should be stored in an opaque bottle to reduce decay during storage. A freshly prepared solution should be used for sanitary purposes such as
cleaning a blood spill. Solutions containing bleach should not be autoclaved. Also be aware that using chlorine compounds to disinfect substances co-contaminated with radioiodine may cause gaseous release of the isotope. Contact with skin should be avoided. Example products: Clidox, Clorox, or other household bleach.

**Iodophors** complexes of iodine and carrier, have good germicidal properties with relatively low toxicity and irritancy. Efficacy has been demonstrated against bacteria including mycobacteria, viruses, and fungi; prolonged contact time may be needed to kill certain fungi and bacterial spores. Example products: Povidine, Betadine.

**Phenolic compounds** are effective against vegetative bacteria, particularly gram positive species, and enveloped viruses but not against spores. Phenolics may be used in combination with detergents for one-step cleaning and disinfection of surfaces. Phenolic disinfectants maintain their activity in the presence of organic material and are generally considered safe, although prolonged exposure of skin may cause irritation. Example products: Vesphene, LpH.

**Quaternary ammonia disinfectants** kill most fungi and vegetative gram positive bacteria but lack efficacy against mycobacteria, spores, and some viruses including adenovirus. Quaternary ammonium compounds generally have low toxicity and irritancy and are relatively inexpensive. Example products: HB Quat, Roccal, Solucide.

It is important to be aware that common laboratory disinfectants can pose hazards to users. Ethanol and quaternary ammonium compounds may cause contact dermatitis. Chlorine in high concentrations irritates the mucous membranes, eyes, and skin. The toxicity of aldehydes limits their usefulness.

Large-volume areas such as fume hoods, biological safety cabinets, or rooms may be decontaminated using gases such as formaldehyde, ethylene oxide, or peracetic acid. These gases, however, must be applied with extreme care. **Only experienced personnel who have the specialized equipment and protective devices to do it effectively and safely should perform gas decontamination.**

**Incineration**

The optimal method of disposal for some types of waste is incineration. Animal carcasses are routinely picked up by the EH&S Department for disposal by this method. Bedding, plastic, and metallic objects must be excluded from packages of animal carcasses. Consult the EH&S Department and/or the [UW Laboratory Safety Guide](#) for more information.

**UV Treatment**

UV light is commonly used for disinfection in a biological safety cabinet and also in clean rooms or areas where PCR is done. Wavelengths below 280 nm cause chemical reactions and therefore have germicidal action. Personnel should avoid exposure to light in this wavelength region since brief exposure can cause erythema, harming skin and eyes. The efficacy of UV light for disinfection is limited by a number of factors. UV light has poor penetration and only vegetative microorganisms directly bathed in it will be affected. UV lights require regular cleaning and monitoring to ensure germicidal activity. Typically, no germicidal benefit is gained from its extended use.
Emergency Plans

No matter how carefully one works, laboratory accidents occur and necessitate emergency response. Emergency plans should be tailored for a given biohazardous situation. The laboratory supervisor should prepare instructions specifying immediate steps to be taken and all personnel should understand basic emergency measures. These instructions should be displayed prominently in the laboratory and periodically reviewed with personnel. No single plan will apply to all situations, but the following general principles should be considered:

- Always know the location of emergency response materials, such as spill kits, extinguishers, eyewashes, safety showers, first aid kits, automated exterior defibrillators (AED), contact numbers or first aid kit.

- In the event of a spill of virulent pathogens, everyone should leave the affected area immediately. Even for apparently small spills, evacuation is important if aerosols were generated. Clothing, if contaminated, should be removed. Exposed skin should be washed thoroughly with soap and water. A splash to the eyes should be treated by flushing with water at a plumbed eyewash for at least 15 minutes.

- If a spill presents immediate danger to people and exceeds the ability of local staff to control it, the event should be reported as an emergency to UW Police.

- Close the laboratory door and post a “No Entry” sign indicating the hazard. Notify the laboratory supervisor and the Office of Biological Safety.

- Determine the necessity and extent of medical treatment for persons exposed to infectious microorganisms.
  - Personnel accidentally exposed via ingestion, skin puncture, or obvious inhalation of an infectious agent should be given appropriate first aid and then seek immediate medical assessment.
  - If necessary, call 9-1-1 or UW Police for transportation to the University Hospital emergency room at any hour.
  - Complete a First Report of Exposure/Report Form online at the OBS website

- Do not reenter the room until aerosols have settled (30 minutes, minimum), and the extent of the hazard and its dissemination has been determined.

- Each person who enters the laboratory for cleanup should wear proper protective clothing.

- Use an appropriately concentrated disinfectant to decontaminate the area. A supply of stock disinfectants should always be available.

- Decontaminate all materials used in cleanup procedures.

In any emergency situation, attention to immediate personal danger overrides containment considerations. With the exception of BSL3 laboratories, properly garbed and masked fire or security personnel are adequately prepared to enter any biological laboratory in an emergency.

Reporting is an additional required step in emergency management. The supervisor should always be notified and a First Report of Exposure/Release Form prepared even in situations that do not involve emergency responders or require immediate medical care. Notify the Biological Safety Officer of any significant problems, violations of the NIH Guidelines, or any significant research-related accidents and illnesses.
Exposure Response  
For any possible or identifiable exposure to a hazardous substance, individuals must seek immediate medical assessment.

- For non-emergency assessments Monday through Friday, 9:00 a.m. to 5:00 p.m., UW employees must go to University Health Service at 333 East Campus Mall (265-5610).
- UW Hospital employees may seek non-emergency assessment at University Hospital Employee Health Service (263-7535).
- For after-hours non-emergency exposure contact University Hospital Emergency Department (262-2398) for assessment
- For emergency medical attention go directly to University Hospital Emergency Department (262-2398); be sure to communicate the exposure event related to the emergency.

PIs are asked in the context of the biosafety protocol to consider the consequences of an accidental exposure to the microbes used in their research and prepare an appropriate response procedure. Organisms that normally are not pathogenic for healthy human adults may become so when the natural barriers to infection are circumvented. The appropriate response for a cutaneous exposure might simply involve thorough washing of the area with soap and water, and for a splash to the eyes flushing with water for 15 minutes. At times it is difficult to ascertain whether an illness is laboratory or community acquired, and you should not discount the possibility that an illness could be related to research activities.

Be prepared to respond to an accidental exposure. The best approach is to have a well-prepared exposure response plan and to provide training to personnel according to this plan. Following are the basic elements of a plan:

- A description of the microbe(s) and the signs and symptoms of infection.
- Distinct characteristics of the laboratory strain(s), such as known antibiotic resistance, transmissibility, atypical tissue tropism, foreign genes that alter pathogenicity, and so forth.
- Recommendations for treatment regarding effective drugs, quarantine, and so forth.
- A test to establish a history of exposure at the start of employment and periodically thereafter may be appropriate for work with a few pathogens such as *Mycobacterium tuberculosis*.
- Completion of a First Report of Exposure/Response form, located online at the OBS website

Transport of Dangerous Goods/Hazardous Materials

In order to protect the public at large, the US Department of Transportation (DOT) regulates the shipping and transportation of hazardous materials (aka dangerous goods) in commerce on roadways, airways and vessels as described in the Code of Federal Regulations Title 49, Parts 171 to 178 (49CFR §171-178). A hazardous material is defined as any substance or material which could adversely affect the safety of the public, handlers or carriers during transportation. All DOT hazardous materials regulated in transport are listed in the 49CFR §172.101 Hazardous Materials Table. Additionally, air transport and international transport of dangerous goods in commerce is regulated by International Air Cargo Organization (ICAO) and International Air
Transport Association (IATA). International and DOT regulations are similar but can vary on some substances; therefore it is crucial to become trained and certified according to both regulatory bodies.

The regulations for shipping hazardous materials apply to all individuals involved in the shipping process, including individuals who:

- Arrange for transport
- Package materials
- Mark and label packages
- Prepare shipping papers
- Handle, load, secure or segregate packages within a transport vehicle.

The Regulations require the individual to receive training in order to become certified to ship hazardous materials. Training must be refreshed at least every 2-3 years (3 years US DOT; 2 years ICAO/IATA) or when regulations significantly change. Regulatory updates commonly occur at the beginning of each year and may or may not be significant; individuals must be cognizant of changes.

EH&S provides Hazardous Materials Shipping certification training through a combination of online and in-person courses. For additional information please see the EH&S Shipping and Transporting Hazardous Materials webpage [http://www.ehs.wisc.edu/hazmatshipping.htm](http://www.ehs.wisc.edu/hazmatshipping.htm).

There are times when it may be impractical to hire a commercial carrier and UW-Madison employees transport biological materials, some of which meet the regulatory definition of a hazardous material, between buildings on the main campus or to outlying areas in an institutional vehicle. This activity does not meet the regulatory definition of transportation in commerce because the UW is a government agency and current regulations allow an exemption from the Hazardous Materials transportation standard. This means that UW employees transporting hazardous materials (except hazardous waste) from building to building and/or on public roadways are not subject to the DOT regulations for personnel training & certification and materials packaging, documentation and labeling.

Although not regulated by federal law, the movement of hazmat on campus or by campus employees must fall UW requirements for hazard communication and containment which virtually mirror those used when employing a commercial carrier. You would not need a dangerous goods declaration, nor would you use the institution’s contract for emergency response.

Use the following steps when transporting hazmat (such as infectious substances or dry ice) without involving a commercial carrier:

1) Use packaging that is appropriate for the material being transported. UN certified packaging is the most sturdy for hazardous materials. Generally a package consists of 3 layers: a primary specimen container, secondary container with absorbent and cushioning material and a rigid outer layer. At least two of the layers should be leak and puncture proof

2) Label the package with emergency contact names and phone numbers

3) Prepare a Safety Data Sheet (SDS) for the pathogen that will accompany the shipment. A library of SDS for Infectious Substances can be found at the Public Health Agency of Canada website [http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php](http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php)
4) Train the worker about nature of the pathogen and emergency procedures and keep a record that the training was performed.

5) Have an emergency response kit (e.g., absorbent, disinfectant, biohazard bags) available at all times during packaging, transport and unpacking of the material.

Movement of Hazardous waste on campus should only be performed by UW EH&S employees or approved contractors. Hazardous waste is regulated by the US Environmental Protection Agency (EPA) and requires specialized training to perform appropriate handling, marking and documentation. Contact EHS Chemical Safety program for assistance and additional information.

**Laboratory Security**

Security commonly refers to safeguarding electronic equipment and personal belongings. Security also needs to be considered in terms of preventing theft of materials from our facilities that have the potential to harm our community.

The UW-Madison Police Department recommends several basic precautions:

- Do not prop doors open; lock doors when no one is present
- Wear visible identification
- Remove sensitive data from the Web
- Report suspicious activities and unauthorized individuals

The degree to which laboratory security is implemented should be commensurate with risk. All laboratories, including those handling only low-risk biological materials under BSL.1 containment practices, must maintain a basic level of security. You should make an effort to know all the people who work in your area, and to greet unknown persons who enter labs and to ask their purpose. According to CDC’s guidance for BSL.1 laboratories, “Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments or work with cultures and specimens is in progress.” Translated into common practice, this statement means that everyone entering a laboratory should have the supervisor’s explicit approval to be there.

Security concerns also extend to hazardous material in storage. Unauthorized persons should not be able to access it. Inventory records are instrumental to determining if there is a discrepancy due to misuse or a security lapse. An easy way to prevent unauthorized access is to lock the laboratory door when the room is unoccupied. For hazardous materials stored outside of the laboratory, such as in a freezer located in a hallway or a common service room, the equipment must be locked at all times.
Biosafety Administration

I. Roles and Responsibilities

Office of Biological Safety
The Office of Biological Safety (OBS) fosters safe laboratory practices and ensures compliance with or implementation of policies, guidelines, or regulations set forth by university administration, the Institutional Biosafety Committee (IBC), and regulatory agencies. This office, under the direction of the Biological Safety Officer, provides many services, including:

- Advises faculty and staff in biosafety matters.
- Provides guidance on recombinant DNA (rDNA) regulations or other aspects of genetic engineering.
- Recommends safe procedures, containment devices, and equipment for all campus activities (research, teaching, diagnostic, and building services) involving biohazards.
- Recommends methods of handling, transporting, decontaminating, and disposing of biohazardous materials.
- Provides advice, in conjunction with the Chemical Safety Program (262-8769), regarding the disposal of sharps (waste capable of creating a puncture wound), biohazardous waste, and biological toxins.
- Provides consultation for containment laboratory/ventilation system design.
- Provides consultation concerning the purchase of biological safety cabinets (BSCs) in cooperation with the Environmental Health Program, which offers a BSC certification program.
- Provides biological safety education and training aids; develops educational and training programs designed to meet the specific biological safety needs of a variety of departments and staff.
- Provides a variety of biological safety references, resources and guidance materials online or in the Office of Biological Safety
- Provides biohazard signs, BSL signs and emergency door cards.
- Provides training and certification for compliance with U.S. Department of Transportation and international regulations for shipping hazardous biological materials.
- Provides administrative support to the Institutional Biosafety Committee (IBC)

Institutional Biosafety Committee (IBC)
The University of Wisconsin–Madison, as an institution receiving research funds from the National Institutes of Health (NIH), is subject to the NIH Guidelines for Research Involving Recombinant DNA Molecules. As mandated by the Guidelines, the Chancellor of the university has appointed an IBC and established, as its administrative office, the Office of Biological Safety (OBS), directed by the Biological Safety Officer. Through the OBS, the IBC transmits its evaluation to the investigator and to UW-Madison Research and Sponsored Programs or university funding committees to satisfy their clearance requirements.

In addition to scrutiny of biosafety protocols, the IBC and OBS assist all faculty and staff in observing safe biological laboratory practices, and endeavor to assure that all biohazardous research is carried out in adequately secure facilities in compliance with all appropriate regulations. The IBC assesses all research elements and determines whether an investigator has adequately addressed safety issues and/or complied with regulations. If necessary, it may require an investigator to take additional safety precautions.
The IBC convenes as necessary (generally the first Wednesday of the month) to review rDNA research and investigations that involve biohazardous materials. To facilitate the review process, investigators should electronically submit their biosafety protocol to the OBS with sufficient lead time, minimally one month before the meeting date. Materials received less than one month before a scheduled IBC meeting will not be considered until the following months’ meeting. Be aware that OBS typically reviews the protocols prior to presentation to the IBC. The OBS will use this time to work with the Principle Investigator (PI) to resolve any protocol issues prior to official IBC review. This process streamlines IBC review and approval during monthly meetings.

Faculty and Staff
University of Wisconsin–Madison faculty and staff are responsible for observing safe practices when handling hazardous biological materials in teaching, research, and clinical laboratories. These materials include pathogenic microorganisms; biological toxins; experimental, biologically active chemicals (carcinogens, mutagens, and teratogens); human blood, body fluids, cells and tissues; Select Agents; exotic and transgenic plants, animals and microbes; recombinant DNA (rDNA); and supplies and equipment used with such substances. In addition, all sharps and hazardous glass and plastic, whether contaminated or not, require careful handling and appropriate disposal.

Principal Investigators (PIs), faculty, and others who supervise people are responsible for the use of proper safety practices by the people they supervise. Everyone is responsible for his or her own adherence to safe work practices and for following safety-related instructions from supervisors. Principal Investigators, instructors, and laboratory supervisors have a special obligation to communicate risks and safety issues in order to instill in their students and laboratory assistants a proactive philosophy concerning safety principles and practices.

An investigator applying for intra- or extramural research support or receiving unsolicited gifts or grants for research involving any potentially hazardous biological material and/or rDNA work that is subject to the NIH Guidelines must obtain clearance for the proposed research. This is done by submitting a biosafety protocol, Biological Materials and Recombinant DNA Protocol, to the OBS. The first page of the form provides administrative information, including a list of grants associated with the protocol. Once registered and assigned a safety committee number (SC#), the protocol is valid for three years. Please note that the signature of the Principal Investigator is always required. The form is available at the Office of Biological Safety website.

Risk assessments of planned experiments should be performed by PIs prior to initiation. The PI’s biosafety protocol can be used for safety training of staff. The criteria for submission of a protocol to the Office of Biological Safety, outlined below in Section II, encompass pathogens (human, plant, and animal), exotic organisms, harmful chemicals administered in vivo or in vitro, select agents and rDNA. Although some rDNA techniques are explicitly exempted, many low-risk experiments are subject to the NIH Guidelines and must therefore be reported for compliance purposes. Investigators must obtain pre-initiation approval from the IBC for rDNA experiments involving genetically engineered products of potential virulence and toxicity or altered drug resistance. In some cases they must also obtain approval from the NIH Recombinant DNA Advisory Committee, or other federal agencies having jurisdiction. However, registration with the IBC is sufficient for proposals of lesser hazard potential.
Investigators contemplating proposals involving rDNA are responsible for familiarizing themselves with the current NIH Guidelines, determining which sections pertain to their experiments, and assessing the appropriate containment levels. The current NIH Guidelines is available electronically as a link from the OBS website. Contact OBS for assistance in interpreting the NIH Guidelines.

The investigator is asked to describe the research protocol, with emphasis on practices and engineering controls employed to contain potentially biohazardous materials. For research involving recombinant DNA techniques, required information includes the source and nature of the DNA and host/vector system(s), and any other relevant details of the experimental protocol. It is important that the investigator identifies potential hazards and describes mitigating procedures or circumstances in sufficient detail such that the OBS and IBC can independently evaluate whether adequate safety measures will be taken.

The Principal Investigator also has the responsibility to notify the Biological Safety Officer of any significant problems, violations of the NIH Guidelines, or any significant research-related accidents and illnesses.

The University

The university must comply with local, state, and federal regulations that apply to biological research and its residuals.

Federal Guidelines: Certain research is subject to federal guidelines and regulations prescribed by the NIH, CDC, the U.S. Department of Agriculture (USDA), the U.S. Environmental Protection Agency, and the U.S. Food and Drug Administration.

Investigators utilizing human blood and other potentially infectious human materials must meet certain requirements. The Occupational Health Officer (263-2177) can assist you in this area.

State Law Regarding rDNA Field Studies: The State of Wisconsin has enacted a law requiring that the Wisconsin Department of Natural Resources or Department of Agriculture, Trade and Consumer Protection (DATCP) be notified of intended field studies of genetically engineered organisms.

Wisconsin Department of Natural Resources Guidelines for Waste Disposal: The DNR has established regulations for the decontamination and elimination of infectious and medical wastes. Appropriate disposal of these wastes is an important aspect of a comprehensive safety program. WI Administrative Codes Chapter NR 526 Medical Waste Management, 2006

Wisconsin Department of Commerce Regulations/OSHA Bloodborne Pathogens Standard: As a public institution, the university must also comply with regulations prescribed by the Wisconsin Department of Commerce, including the Bloodborne Pathogens Standard mandated by the Occupational Safety and Health Administration (OSHA).

II. Biosafety Protocol Registration Process

As a major research institution, the UW-Madison provides assurances that its sponsored research activities are in compliance with state and federal regulations and guidelines. In this context, the Institutional Biosafety Committee (IBC) reviews research activities involving biologically hazardous materials and/or recombinant DNA molecules/organisms.

Biosafety protocols must be submitted to the Office of Biological Safety (OBS) for research activities involving the following:
- Microbiological agents infectious to humans and/or animals.
- Exotic plants, animals, and microbes (e.g., nonindigenous plants or insect pathogens, or biological control agents).
- Potentially infectious materials derived from humans (e.g., established cell lines) and from animals, including their blood, tissues, and cell lines, for which a reasonable potential for transmission of zoonotic agents exists, e.g., wild-trapped animals, sheep, and rhesus macaques.
- Potentially hazardous chemicals administered in vivo or in vitro to induce a biological outcome (e.g., carcinogens, mutagens, teratogens, drugs, and toxins).
- Select agents. These microbes and toxins are federally regulated due to their threat to public health and safety. The list of select agents is available at the OBS website and upon request from OBS.

- Recombinant DNA molecules and recombinant DNA-containing organisms or cell cultures which are subject to the NIH Guidelines for Research Involving Recombinant DNA Molecules. Contact OBS about requirements for protocols involving human gene therapy trials. Many rDNA experiments are considered to be low-risk yet still are subject to the Guidelines.

- Biological toxins harmful to humans, animals or plants.

Protocol descriptions must be submitted every three years to ensure that protocols remain current with research activities. Protocols will be considered inactive after three years unless an updated protocol is submitted for review. We encourage protocol consolidation, even for separate projects funded by multiple agencies, to facilitate a comprehensive risk assessment and to reduce the administrative burden.

The biosafety protocol registration form is available at the OBS website in formats suitable for PC and Macintosh word processors. Investigators are asked to use the current version of the form. Keep an electronic copy of the protocol so that it will be easy to add project-specific information regarding grants, materials, and locations. The website also provides access to other important biosafety information.

General points of consideration when writing a biosafety protocol:

**Amendments**

The protocol, once registered, is valid for three years and may be amended during that period. Grant submissions that relate to the existing protocol may be added simply by entering this information in Section I of the form (Core Registration Information) and resubmitting this single page. Additional information about locations and/or research elements can be entered into the existing protocol using a distinctive font (e.g., bold).

**Confidentiality**

The OBS handles protocols in a manner that maintains their confidentiality in order to protect intellectual and proprietary information.

**Enumerating Details**

It is not necessary to provide the details for every permutation of a research element, such as gene constructs or pharmaceutical compounds. Instead, group elements by categories associated with risk and provide at least one specific example. Be sure your biosafety protocol and animal care protocol are consistent with the materials described.
Expiration Date
Biosafety protocols expire three years from the date they are registered. OBS will send a reminder to the Principal Investigator prior to the expiration date and again after the fact if no response to the notice was received.

Funding Status
The funding status of a research project is not relevant in this context. A biosafety protocol should encompass all potentially hazardous aspects of a biological research program, whether currently funded or not. Include Material Transfer Agreements. To avoid unnecessary delays, list applications at the time they are submitted, whether funding is awarded or not. When submitting a protocol for renewal (three-year update), list continuing and pending awards.

Human Gene Therapy Trials
Human gene therapy (HGT) trials must be reviewed by the IBC in advance of patient enrollment. Supplement the protocol form with responses to the points of consideration outlined by the NIH Guidelines, Appendix M. Also submit the Investigational Drug Brochure, Study Protocol, and informed consent form.

IBC Review
Protocols that involve activities subject to the NIH Guidelines will be reviewed by the IBC, as are other protocols identified as having significant risk. Approval of a protocol by the IBC may have contingencies or a request for additional information that must be satisfied before the registration of the protocol is finalized. Approval, however, could be denied or deferred until specific issues are addressed.

Inventory
Investigators are encouraged to keep an inventory of all potentially hazardous materials in their possession. For those working with Select Agents, an inventory of agents and quantities is a requirement. The appendix at the end of the biosafety protocol form should be used to list pathogens, toxins, and regulated organisms that are stored and not actively used in current research projects.

Locations
All locations where hazardous materials will be used should be listed in the protocol. Include the facilities of UW-Madison collaborators and service centers that will handle hazardous or potentially hazardous materials covered by the protocol. Inclusion of biosafety cabinet (BSC), fume hoods and autoclaves should also be listed.

PI Status
The Principal Investigator and the Co-PIs must be UW faculty or staff who have PI status by virtue of their position or by having been granted this status by the Graduate School.

Protocol Number
The OBS assigns a unique number (SC#) to each protocol submitted and reviewed. This number is retained for the three-year period that the protocol is considered valid; amendments to the protocol retain the number assigned to the original submission.

Protocol Submission Deadline
Protocols that will be reviewed by the IBC should be submitted to OBS one month prior to an IBC meeting, which tentatively is scheduled for the first Wednesday of each month. Materials received less than one month before a scheduled IBC meeting will not be considered until the following meeting. The schedule is posted at the OBS website.

Signature
The Principal Investigator must sign the protocol and any amendments to it. Information
submitted electronically without a signature will be accepted if it comes directly from a PI's email address or the PI is copied on the email request.

III. Lab Visits
Visits to facilities are conducted to ensure safe and compliant conduct of biological research. Additional goals include

- to meet the needs of researchers for guidance on biosafety and regulatory issues;
- to facilitate communication between staff and OBS;
- to discuss facility issues;
- to ensure that our records accurately reflect ongoing research activities;

These visits are designed to be informational, instructional and collegial. The goal for OBS lab visits is to develop a relationship with our laboratory PIs, graduate students, technicians and laboratory staff and support staff. Through this relationship the OBS can be a resource for biosafety protocols, lab safety practices, regulatory information and updates, training and guidance. These elements are essential to foster growth in our exemplary research institution.

IV. Training

Biosafety
Biological safety training is offered by OBS through Learn@UW online courses, in-person classes and online reference materials. Most training courses are optional based on your laboratory needs or work position; however some are required. Required courses are clearly marked in the list below and on the OBS website Training tab.

Maintain a record of training activities, including not just formal classroom sessions but also topics covered during staff meetings and one-on-one mentoring.

Learn@UW Training Modules

Biosafety 101: Building Biosafety into Your Research – Risk Assessment
**Required** for all personnel working in labs under a Biological Safety Protocol
Renew training every 5 years.

Biosafety 102: Bloodborne Pathogens in the Laboratory
Meets Bloodborne Pathogen (BBP) training requirement for staff working with human source materials in the laboratory.

Biosafety 103: Building Biosafety into Your Research - Exposure Response
Guidance training for any personnel working in labs with biohazardous materials.

Biosafety 104: Building Biosafety into Your Research - Safe Use of Sharps
**Required** for all personnel working in labs under a Biological Safety Protocol
Renew training every 5 years.

Biosafety 105: Building Biosafety into Your Research - Biosafety Cabinet Use
Guidance training for any personnel working in labs using Biological Safety Cabinets (BSCs).

Biosafety 106: Autoclave Use – Safety and Efficacy
Guidance training for any personnel working in labs using autoclaves.

Biosafety 107: Centrifuge Safety
Guidance training for any personnel working in labs using laboratory centrifuges.

**Biosafety 201: NIH Guidelines**
Required for all personnel working in labs under a Biological Safety Protocol
Renew training every 5 years.

**Biological Hazardous Materials Shipping Certification Training**

**Biosafety 205, 206, 207**
Hazardous materials, capable of posing an unreasonable risk to health, safety, and property, are commonplace in university facilities. When hazardous materials are transported in commerce, complex federal and international regulations must be followed. Seemingly minor technical violations can result in major fines, while more serious violations can endanger the public.

The U.S. Department of Transportation (US DOT) and International Air Transport Association (IATA), require all persons involved in shipping hazardous materials in commerce to be trained and certified in proper handling of these materials. Activities for which training is required include preparation of shipping papers, packing, package marking, package labeling, package handling/loading and supervision of such activities.

The OBS training modules Biosafety 205 (on Learn@UW) and 206 (a hands-on workshop) provide initial certification training according to the Dangerous Goods/Hazardous Materials Regulations of the IATA and US DOT, focused specifically on shipping infectious substances and other biological materials, as well dry ice. Certification is valid for two years under these.

Renewal of certification can be accomplished by completion of a shorter Recertification course, Biosafety 207, also available on Learn@UW.

**Biosafety 205: Hazardous Materials Shipping: Infectious and Biological substances**
(Bio-HazMat) - Required for Initial Certification UW Madison Personnel who ship and receive infectious and biological substances

**Biosafety 206: Hazardous Materials Shipping: Packaging Workshop**
Required for Initial Certification for UW Madison Personnel who ship and receive infectious and biological substances

**Biosafety 207: Recertification for Bio Hazardous Materials Shipping**
Required for Renewal of Certification of UW Madison Personnel who ship and receive infectious and biological substances

**Safety for Personnel with Animal Contact**
Required for all personnel with animal contact.
Overview of occupational health and safety topics including animal contact safety, zoonoses, allergies & asthma, ergonomics, medical concerns, immunizations, injury and exposure protocols, syringe use guidelines, and personal protective equipment.

**Agricultural Safety**
Agricultural Safety is an online course designed for individuals working and learning in agricultural settings.
**Online Training Materials (not on Learn@UW)**

**Assorted Biosafety Research and Laboratory Specialized Topics**  
May Include: Liquid Nitrogen Safety, Biosafety Level Review, Viral Vector Safety, or others

**Biosafety Training Materials for Select Agent Program Labs**  
Required for Select Agent Program staff; restricted, password-protected access portal through the Select Agent Program website.

**Relevant Training Materials (Non-OBS )**

**Export Control for Biological Agents**  
*Strongly Recommended* for anyone shipping biological materials outside the U.S. Important federal regulatory information for exporters presented by the UW-Madison Export Control Officer.

**Body Fluid Spill Cleanup Procedure** (for human body fluids)  
For UW Madison personnel responsible for human body fluid spill clean-up. Administered through the Occupational Health Office Bloodborne Pathogen Safety program.

**Syringe Use Guidelines**  
For UW Madison personnel who use syringes for the manipulation of human body fluids. Administered through the Occupational Health Office Bloodborne Pathogen Safety program.

**Respiratory Protection**  
Applicable to UW Madison Personnel using respirators (training is mandatory if work conditions require use of respiratory protection). Administered through the Occupational Health Office.

**LN2 Safety**  
Applicable to UW Madison personnel who work with or around liquid nitrogen (LN2)

**Chemical Safety**  
Training is available through the EH&S Chemical Safety Office.

**Bloodborne Pathogens Program – OSHA Non-laboratory**  
Training is available through the UHS EOH.

**Radiation Safety**  
Training is available through the EH&S Radiation Safety Office.
BioSide Lines

Newsletter of the Office of Biological Safety

BioSide Lines is a quarterly newsletter publication of the University of Wisconsin – Madison Office of Biological Safety that supplements the guidance provided in this biosafety manual.

BioSide Lines covers topics of current interest regarding laboratory safety, training, policy and regulatory compliance. The newsletter is distributed electronically to a mailing list which includes PIs with registered biosafety protocols, individuals who have completed previous safety trainings, UW-Madison departments involved with biological research and others.

If you would like to be included on the BioSide Lines mailing list, please contact the OBS at biosafety@fpm.wisc.edu. The current and previous issues are available at the OBS website.
Appendix A: Classification of Human Pathogens on the Basis of Hazard

Biological agents that are known to infect humans are classified according to risk groups. The following listing of the more commonly encountered agents is reproduced from Appendix B of the NIH Guidelines. Included are representative genera and species known to be pathogenic; it is not meant to be all-inclusive. Those agents not listed in RG2 through RG4 are not automatically or implicitly classified in RG1.

**Risk Group 1 (RG1) Agents**

RG1 agents are not associated with disease in healthy adult humans. Examples of RG1 agents include asporogenic *Bacillus subtilis* or *Bacillus licheniformis*; adeno-associated virus (AAV) types 1 through 4; and recombinant AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and are produced in the absence of a helper virus.

A strain of *Escherichia coli* is an RG1 agent if it (1) does not possess a complete lipopolysaccharide (i.e., lacks the O antigen); and (2) does not carry any active virulence factor (e.g., toxins) or colonization factors and does not carry any genes encoding these factors.

Those agents not listed in Risk Groups (RGs) 2, 3 and 4 are not automatically or implicitly classified in RG1; a risk assessment must be conducted based on the known and potential properties of the agents and their relationship to agents that are listed.

**Risk Group 2 (RG2) Agents**

RG2 agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available.

**Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia**

- *Acinetobacter baumannii* (formerly *Acinetobacter calcoaceticus*)
- *Actinobacillus*
- *Actinomyces pyogenes* (formerly *Corynebacterium pyogenes*)
- *Aeromonas hydrophila*
- *Amycolata autotrophica*
- *Arcanobacterium haemolyticum* (formerly *Corynebacterium haemolyticum*)
- *Arizona hinshawii* - all serotypes
- *Bacillus anthracis*
- *Bartonella henselae*, *B. quintana*, *B. vinsonii*
- *Bordetella* including *B. pertussis*
- *Borrelia recurrentis*, *B. burgdorferi*
- *Burkholderia* (formerly *Pseudomonas* species) except those listed in RG3
- *Campylobacter coli*, *C. fetus*, *C. jejuni*
- *Chlamydia psittaci*, *C. trachomatis*, *C. pneumoniae*
- *Clostridium botulinum*, *C. chauvoei*, *C. baemolyticum*, *C. histolyticum*, *C. novyi*, *C. septicum*, *C. tetani*
- *Corynebacterium diphtheriae*, *C. pseudotuberculosis*, *C. renale*
- *Dermatophilus congolensis*
- *Edwardsiella tarda*
- *Erysipelothrix rhusiopathiae*
- *Escherichia coli* - all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen, including *E. coli* O157:H7
- *Haemophilus ducreyi*, *H. influenzae*
- *Helicobacter pylori*
- *Klebsiella* - all species except *K. oxytoca* (RG1)
- *Legionella* including *L. pneumophila*
- *Leptospira interrogans* - all serotypes
- *Listeria*
- *Moraxella*
- *Mycobacterium* (except those listed in RG3) including *M. avium* complex, *M. asiaticum*, *M. boris* BCG vaccine strain, *M. chelonei*, *M. fortuitum*, *M. kansasii*, *M. leprae*, *M. malmoense*, *M. marinum*, *M. paratuberculosis*, *M. scrofulaceum*, *M. simiae*, *M. szulgai*, *M. ulcerans*, *M. xenopi*
- *Mycoplasma*, except *M. mycoides* and *M. agalactiae* which are restricted animal pathogens
- *Neisseria gonorrhoeae*, *N. meningitidis*
- *Nocardia asteroides*, *N. brasiliensis*, *N. otitidiscaviarum*, *N. transvalensis*
- *Rhodococcus equi*
- *Salmonella* including *S. arizonae*, *S. cholerasuis*, *S. enteritidis*, *S. gallinarum-pullorum*, *S. melagridis*, *S. paratyphi*, *A, B, C*, *S. typhi*, *S. typhimurium*
- *Shigella* including *S. boydii*, *S. dysenteriae*, type 1, *S. flexneri*, *S. sonnei*
- *Sphaerophorus necrophorus*
- *Staphylococcus aureus*
- *Streptobacillus moniliformis*
- *Streptococcus* including *S. pneumoniae*, *S. pyogenes*
- *Treponema pallidum*, *T. carateum*
- *Vibrio cholerae*, *V. parahemolyticus*, *V. vulnificus*
- *Yersinia enterocolitica*

**Risk Group 2 (RG2) - Fungal Agents**

- *Blastomyces dermatitidis*
- *Cladosporium bantianum*, *C. (Xylolypha) trichoides*
- *Cryptococcus neoformans*
- *Dactylaria galapava* (Ochroconis gallopavum)
- *Epidermophyton*
- *Exophiala* (*Wangiella*) *dermatitidis*
- *Fonsecaea pedrosoi*
- *Microsporum*
- *Paracoccidioides brasiliensis*
- *Penicillium marneffei*
- *Sporobothrix schenckii*
- *Trichophyton*

**Risk Group 2 (RG2) - Parasitic Agents**

- *Ancylostoma* human hookworms including *A. duodenale*, *A. ceylanicum*
- *Ascaris* including *Ascaris lumbricoides suum*
- *Babesia* including *B. divergens*, *B. microti*
- *Brugia* filaria worms including *B. malayi*, *B. timori*
- *Coccidia*
- *Cryptosporidium* including *C. parvum*
- *Cysticercus cellulosae* (hydatid cyst, larva of *T. solium*)
- *Echinococcus* including *E. granulosis*, *E. multilocularis*, *E. vogeli*
- *Entamoeba histolytica*
- *Enterobius*
- *Fasciola* including *F. gigantica*, *F. hepatica*
- *Giardia* including *G. lamblia*
- *Heterophyes*
- *Hymenolepis* including *H. diminuta*, *H. nana*
- *Iospora*
- *Leishmania* including *L. braziliensis*, *L. donovani*, *L. ethiopia*, *L. major*, *L. mexicana*, *L. peruviana*, *L. tropica*
- *Loa loa* filaria worms
- *Microsporidium*
- *Naegleria fowleri*
- *Necator* human hookworms including *N. americanus*
- *Onchocerca* filaria worms including, *O. volvulus*
- *Plasmodium* including simian species, *P. cynomologi*, *P. falciparum*, *P. malariae*, *P. ovale*, *P. vivax*
- *Sarcocystis* including *S. suis hominis*
- *Schistosoma* including *S. haematobium*, *S. intercalatum*, *S. japonicum*, *S. mansoni*, *S. mekongi*
- *Strongyloides* including *S. stercoralis*
- *Taenia solium*
- *Toxocara* including *T. canis*
- *Toxoplasma* including *T. gondii*
- *Trichinella spiralis*
- *Trypanosoma* including *T. brucei brucei*, *T. brucei gambiense*, *T. brucei rhodesiense*, *T. cruzi*
- *Wuchereria bancrofti* filaria worms

**Risk Group 2 (RG2) - Viruses**

Adenoviruses, human - all types
- Alphaviruses (Togaviruses) - Group A Arboviruses
  - Eastern equine encephalomyelitis virus
  - Venezuelan equine encephalomyelitis vaccine strain TC-83
  - Western equine encephalomyelitis

Arenaviruses
- Lymphocytic choriomeningitis virus (non-neurotropic strains)
- Tacaribe virus complex
- Other viruses as listed in the reference source (see NIH Guidelines Section V-C, Footnotes and References of Sections I through IV)

Bunyaviruses
- Bunyamwera virus
- Rift Valley fever virus vaccine strain MP-12
- Other viruses as listed in the reference source (see NIH Guidelines Section V-C, Footnotes and References of Sections I through IV)

Caliciviruses

Coronaviruses

Flaviviruses (Togaviruses) - Group B Arboviruses
- Dengue virus serotypes 1, 2, 3, and 4
- Yellow fever virus vaccine strain 17D
- Other viruses as listed in the reference source (see NIH Guidelines Section V-C, Footnotes and References of Sections I through IV)

Hepatitis A, B, C, D, and E viruses

Herpesviruses - except Herpesvirus simiae (Monkey B virus) (see Risk Group 4)
  - Cytomegalovirus
  - Epstein Barr virus
  - *Herpes simplex* types 1 and 2
  - *Herpes zoster*
  - Human herpesvirus types 6 and 7

Orthomyxoviruses
  - Influenza viruses types A, B, and C
  - Other tick-borne orthomyxoviruses as listed in the reference source (see NIH Guidelines Section V-C, Footnotes and References of Sections I through IV)

Papovaviruses
  - All human papilloma viruses

Paramyxoviruses
  - Newcastle disease virus
  - Measles virus
  - Mumps virus
  - Parainfluenza viruses types 1, 2, 3, and 4
  - Respiratory syncytial virus

Parvoviruses
  - Human parvovirus (B19)

Picornaviruses
  - Coxsackie viruses types A and B
  - Echoviruses - all types
  - Polioviruses - all types, wild and attenuated
  - Rhinoviruses - all types

Poxviruses
  - all types except Monkeypox virus (see Risk Group 3) and restricted poxviruses including Alastrim, Smallpox, and Whitepox

Reoviruses
  - all types including Coltivirus, human Rotavirus, and Orbivirus (Colorado tick fever virus)

Rhabdoviruses
  - Rabies virus - all strains
  - Vesicular stomatitis virus - laboratory adapted strains including VSV-Indiana, San Juan, and Glasgow

Togaviruses (see Alphaviruses and Flaviviruses)
  - Rubivirus (rubella)

**Risk Group 3 (RG3) Agents**

RG3 agents are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available.

**Risk Group 3 (RG3) - Bacterial Agents Including Rickettsia**
  - *Bartonella*
- *Brucella* including *B. abortus, B. canis, B. suis*
- *Burkholderia (Pseudomonas) mallei, B. pseudomallei*
- *Coxiella burnetii*
- *Francisella tularensis*
- *Mycobacterium bovis* (except BCG strain, see Risk Group 2), *M. tuberculosis*
- *Pasteurella multocida* type B - "buffalo" and other virulent strains
- *Yersinia pestis*

**Risk Group 3 (RG3) - Fungal Agents**
- *Coccidioides immitis* (sporulating cultures; contaminated soil)
- *Histoplasma capsulatum, H. capsulatum var. dubois*

**Risk Group 3 (RG3) - Parasitic Agents - None**

**Risk Group 3 (RG3) - Viruses and Prions**

**Alphaviruses (Togaviruses) - Group A Arboviruses**
- *Semliki Forest virus*
- *St. Louis encephalitis virus*
- *Venezuelan equine encephalomyelitis virus* (except the vaccine strain TC-83, see RG2)
- Other viruses as listed in the reference source (see NIH Guidelines Section V-C, Footnotes and References of Sections I through IV)

**Arenaviruses**
- *Flexal*
- *Lymphocytic choriomeningitis virus* (LCM) (neurotropic strains)

**Bunyaviruses**
- *Hantaan virus including Hantaan virus*
- *Rift Valley fever virus*

**Flaviviruses (Togaviruses) - Group B Arboviruses**
- *Japanese encephalitis virus*
- *Yellow fever virus*
- Other viruses as listed in the reference source

**Poxviruses**
- *Monkeypox virus*

**Prions**
- *Transmissible spongiform encephalopathies (TSE) agents* (Creutzfeldt-Jacob disease and kuru agents)

**Retroviruses**
- *Human immunodeficiency virus* (HIV) types 1 and 2
- *Human T cell lymphotropic virus* (HTLV) types 1 and 2
- *Simian immunodeficiency virus* (SIV)

**Rhabdoviruses**
- *Vesicular stomatitis virus*
**Risk Group 4 (RG4) Agents**

RG4 agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available.

**Risk Group 4 (RG4) - Bacterial Agents - None**

**Risk Group 4 (RG4) - Fungal Agents - None**

**Risk Group 4 (RG4) - Parasitic Agents - None**

**Risk Group 4 (RG4) - Viral Agents**

- **Arenaviruses**
  - Guanarito virus
  - Lassa virus
  - Junin virus
  - Machupo virus
  - Sabia

- **Bunyaviruses (Nairovirus)**
  - Crimean-Congo hemorrhagic fever virus

- **Filoviruses**
  - Ebola virus
  - Marburg virus

- **Flaviruses (Togaviruses) - Group B Arboviruses**
  - Tick-borne encephalitis virus complex including Absetterov, Central European encephalitis, Hanzalova, Hypr, Kumlinge, Kyasanur Forest disease, Omsk hemorrhagic fever, and Russian spring-summer encephalitis viruses

- **Herpesviruses (alpha)**
  - Herpesvirus simiae (Herpes B or Monkey B virus)

- **Paramyxoviruses**
  - Equine morbillivirus

Hemorrhagic fever agents and viruses as yet undefined

**Animal Viral Etiologic Agents in Common Use**

The following list of animal etiologic agents is appended to the list of human etiologic agents. None of these agents is associated with disease in healthy adult humans; they are commonly used in laboratory experimental work. A containment level appropriate for RG1 human agents is recommended for their use. For agents that are infectious to human cells, e.g., amphotropic and xenotropic strains of murine leukemia virus, a containment level appropriate for RG2 human agents is recommended.

- **Baculoviruses**
- **Herpesviruses**
  - Herpesvirus ateles
  - Herpesvirus saimiri
  - Marek’s disease virus
  - Murine cytomegalovirus

- **Papovaviruses**
- Bovine papilloma virus
- Polyoma virus
- Shope papilloma virus
- Simian virus 40 (SV40)

Retroviruses
- Avian leukosis virus
- Avian sarcoma virus
- Bovine leukemia virus
- Feline leukemia virus
- Feline sarcoma virus
- Gibbon leukemia virus
- Mason-Pfizer monkey virus
- Mouse mammary tumor virus
- Murine leukemia virus
- Murine sarcoma virus
- Rat leukemia virus

Appendix B-V-1. Murine Retroviral Vectors

Murine retroviral vectors to be used for human transfer experiments (less than 10 liters) that contain less than 50% of their respective parental viral genome and that have been demonstrated to be free of detectable replication competent retrovirus can be maintained, handled, and administered, under BSL1 containment.
Appendix B:
BSL3 Manual: Points to Consider

The purpose of preparing a BSL3 manual is to provide detailed procedures that staff follow while working in your facility under BSL3 containment. The manual should be specific to the facility, agents, and procedures used. By providing details about the precautions and procedures actually used, OBS can provide feedback and engage in discussions regarding possible improvements.

The manual should be written so that it serves as a training tool for employees. The instructions must be explicit. Vague statements such as “appropriate steps must be followed” are not acceptable. The manual also serves to meet the requirement stated in Biosafety in Microbiological and Biomedical Laboratories that a manual specific to the agents/procedures/facilities is prepared, and should satisfy the Institutional Biosafety Committee that hazards are mitigated.

Two documents that should be consulted are
- CDC/NIH, Biosafety in Microbiological and Biomedical Laboratories, 5th ed., Section III  
  http://www.cdc.gov/biosafety/publications/bmbl5/
- NIH/OBA Guidelines for Research Involving Recombinant DNA Molecules, Appendix G-II-C  

The following topics should be considered. Some may not apply to your situation. This list is not all inclusive.

Procedures for Entry
- Hazard communication signage posted
- Who is allowed into the facility? How is a shared facility managed?
- Physical security that limits access
- Entry requirements (immunization, fitness to wear a respirator, etc.)
- Personal protective equipment that is to be worn (may be different for lab vs. animal rooms and vary by procedures to be conducted) and the procedure to put it on
- Respirator(s): What kind is used? Who does fit testing, medical evaluation to wear a respirator, and training? If reused, how is it cleaned/stored? For what procedures should it be used (lab and animal procedures)?
- Training requirements to work in the BL3 facility (how often, how extensive, documentation, demonstration of proficiency, etc.)

Routine Procedures/Precautions
- What containment methods are used for various procedures?
- Is plastic-ware substituted for glass?
- Handling of sharps (generally discouraged in BL3 facilities)
- Precautions used when aerosols might be generated outside a BSC (e.g., centrifugation and flow cytometry)
- Precautions/containment used if mixed hazards present (e.g., biological and chemical)
- Are staff allowed to work alone at night and weekends (generally discouraged)?
- Is a hand-washing/hands-free sink available?
- Are ventilation monitoring systems checked and readings recorded?
- Precautions used for vacuum lines (e.g., filters or disinfectant traps)
- Autoclave efficacy testing (how often and method)
- Precautions used during necropsy
- Housekeeping procedures: How often is each task done? Who is responsible?

**Routine Maintenance and Repair Procedures**
- Surface cleaning: How often it is done? What disinfectant at what concentration is used?
- Who washes the floor and how often? If tile floor, how is impervious surface maintained and by whom?
- Who is responsible for flushing the eyewash?
- Procedures for allowing custodians and repair workers into facility: Are they accompanied at all times? Are they informed of hazards and what they should stay away from? Will their equipment need to be surface decontaminated prior to exit?
- Procedures to be used if equipment needs to be sent out for repairs

**Equipment/Facility Certification, Maintenance, and Testing**
- Annual shutdown of the facility to certify, repair, and test facility and equipment
- Procedures for decontamination prior to facility work beginning
- Annual certification of BSCs and facility HEPA filters (required)
- Annual inspection, maintenance/repair, and testing of facility to verify containment: interior surfaces (floors, walls, ceiling, bench tops), plumbing, electrical, specialized HVAC system and controls

**Procedures for Exit**
- Details for removing personal protective equipment, disinfecting and/or discarding it
- Waste removal/disposal
- Laundering of lab coats
- Precautions used if/when materials are removed from the BL3 facility (e.g., papers and viable or fixed samples)

**Animal Handling**
- Assignment of responsibilities: animal care staff or researchers?
- Precautions for administering pathogens to animals
- Type of cages/cage systems used
- Bedding changes
- Cage cleaning: autoclaved prior to washing?
- Animal and animal waste disposal: autoclaved and/or incinerated?

**Emergency Response**
- What constitutes an emergency and what are the procedures for dealing with it?
- What if your BSC, incubator, or centrifuge quits?
- What if the room pressure alarm goes off the lights go out, or there is a needle-stick?
- What if an infected animal gets loose?
- Other worst case scenarios?
- Who should be contacted?
- Provide a hierarchical contact list that your personnel could use for notification when you are unavailable in case there is an incident such as suspicion of exposure.
- Should individuals carry information regarding the nature of the pathogen that they handle?
- What does it mean when an alarm sounds? How should staff respond to an alarm?
- Provide a copy of the spill procedure(s) to be posted in the facility.
- Where are the nearest eyewash and emergency shower located?
Useful References

Note: URLs of remote sites change frequently. The OBS website has a more current set of links, or you may need to search from the root directory of each organization.


Public Health Agency of Canada, Material Safety Data Sheets (MSDS) for Infectious Substances http://www.phac-aspc.gc.ca/msds-frss/index-eng.php


## Contact Information

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