

Office of Biological Safety
Safety Department
University of Wisconsin–Madison
30 North Murray Street
Madison, WI 53715
<http://www.fpm.wisc.edu/biosafety>

Issued July 1982. Revised June 1986, August 1988, August 1992, October 1994,
May 1999, November 2004.

Additional copies of this book can be obtained from the Office of Biological Safety,
30 North Murray Street, Madison, WI 53715. 608-263-2037.

Cover Emblem: Universal Biohazard Symbol

Signifies actual or potential contamination of equipment, rooms, materials,
or animals by viable infectious agents.

Table of Contents

Introduction	2
General Principles of Biological Safety	2
Risk Assessment	2
Clinical and Pathological Specimens	4
Cultures	5
Animals	5
Plant Biocontainment	6
Laboratory Exposure	7
Health Status	8
Biohazard Containment	9
Practices and Procedures	9
Personal Protective Equipment	10
Engineering Controls	11
Disposal of Wastes from Biological Laboratories	18
What Is Infectious Waste?	18
Methods of Decontamination	20
Emergency Plans	23
Exposure Response	24
Transport of Dangerous Goods	24
Laboratory Security	25
Biosafety Administration	26
Roles and Responsibilities	26
Biosafety Protocol Registration Process	29
Lab Visits	31
Training	31
BioSide Lines	33
Useful References	34
Appendix A, Classification of Human Pathogens on the Basis of Hazard	35
Appendix B, BL3 Manual: Points to Consider	42
Contact Information	(back cover)
TABLES	
Table 1. Relationship of Risk Groups to Biosafety Levels, Practices, Facilities, and Equipment	8
Table 2. Summary of Facility Standards Recommended for Biosafety Levels	13
Table 3. Ventilation Equipment	15

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 infectious or toxic microorganisms, potentially infectious human substances, 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 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, 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, and molecular biology. 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 Office of Biological Safety.

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) correspond to biosafety levels (BL1 through BL4), 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 biological safety data sheets provided by Health Canada are another excellent source of information about human 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*, Adenovirus, *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 standard laboratory facilities and standard microbiological practices, whereas those in RG4 require maximum containment. 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 plants, which are not categorized like human pathogens into risk groups. 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, 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 with other pathogens when aerosol-generating procedures are done and when high concentrations or large volumes are used. Appropriate precautions can be implemented to avoid such exposures.

Clinical and Pathological Specimens

Every specimen from people or animals may contain infectious agents. Human specimens are 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 enroll in the campus 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, which is 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 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
- 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 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, 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 low incidence 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. BL2 containment conditions should be 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 tissue should also be handled using BL2 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 using a lab coat and eye protection when handling animals and their bedding; gloves and respiratory protection may be recommended when specific conditions such as animal allergens 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 high level of hazard, requiring that stringent procedures be followed to guard against Herpes B virus (*Cercopithecine herpesvirus 1*). Even in the absence of any other hazard, 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 unintentionally, 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 or primate cells, whether newly isolated or well established, into immunocompromised animals could result in propagation of pathogens that would be suppressed in the normal host. BL2 containment must be applied to mitigate against such risks and also to prevent spread of animal pathogens within a research colony.

Some animals may have been 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 that is contaminated with certain particularly hazardous substances must be decontaminated prior to disposal; the Chemical and Radiation Protection Office should be consulted for this determination.

Plant Biocontainment

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 (BL1-P through BL4-P) are utilized (see Appendix P, NIH *Guidelines for Research Involving Recombinant DNA Molecules*). BL1-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. BL2-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. BL3-P and BL4-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.

BL1-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. BL1-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. BL2-P and BL3-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 is 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

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

^a OSHA Lab Standard

Source: Modified from WHO *Laboratory Biosafety Manual* (2003)

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. These practices and procedures provide the foundation for the more restrictive containment of RG3 organisms, which is not covered in this manual. Specialized facilities and rigorous attention to procedures that control the biohazards are required for the conduct of research under BL3 containment, which must be described in a biosafety manual that is specific to the agents, facilities, and activities. Points to consider in writing the BL3 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 personal items such as coats, boots, bags, and books 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. These signs are available from the Office of Biological Safety. Identify the agents in use and the appropriate emergency contact personnel.
- ✓ Wear a fully fastened laboratory coat, gloves, and eye protection when working

with infectious agents or potentially hazardous materials, including human blood and body fluids.

- ✓ 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 and the location of cleanup equipment.
- ✓ 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, Tyvek suits, 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 Safety Department offers prescription safety glasses at minimal cost to help people 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 because of use of good microbiological techniques. 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. With the exception of clinical specimens with *Mycobacterium tuberculosis*, recommendations for respirators are not documented for work with pathogens since acceptable exposure levels have not been determined.

One of the issues regarding respiratory protection is that, if used improperly, the user has a false sense of security. A surgical mask, which has poor fit to the contours of the face, provides minimal protection against large particles and is 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 is used when respiratory protection is critical for work with highly pathogenic microbes and 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 Safety Department 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 annually and verified to retain microorganisms.

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).

Table 2. Summary of Facility Standards Recommended for Biosafety Levels

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

--, not applicable or needed.

Existing facilities that do not meet these recommendations should plan to address deficiencies during future maintenance or remodeling. Contact the Office of Biological Safety for assistance.

^a Required by current Wisconsin Administrative Code.

Separated into classes and types: Class I, Class II (Type A1/A2/B1/B2), Class III (glovebox, 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 Biohazards: Selection, Installation, and Use of Biological Safety Cabinets*, published by the CDC and NIH.

Laminar flow clean benches are not biological safety cabinets and should never be used for work with potentially hazardous biological, radioactive, 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 superclean 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

Table 3. Ventilation Equipment

Device	Protection	Direction of Airflow (feet/min)	Application/Airflow Pattern	Appropriate for Some Uses of Volatile Toxic Chemicals and Radionuclides
Chemical fume hood	Personnel only	Inward (100)	A completely exhausted, unfiltered device used for work with chemical hazards, minimizing exposure to personnel.	Acceptable
Clean air device Clean bench	Product only	Outward (100)	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).	Not Acceptable
Animal transfer station	Product and 70% personnel	Inward	A HEPA-filtered device used to transfer animals from dirty to clean cage, minimizing exposure to animal and personnel.	Not Acceptable
Bedding dump station	Personnel and environment	Inward	A HEPA-filtered device used to capture airborne particulate when disposing of waste bedding from animals, minimizing exposure to personnel.	Not Acceptable
BSC Class I	Personnel and environment	Inward (100-200)	Effectively a fume hood with filtered exhaust. HEPA filtered exhaust air passes through a dedicated duct system to the outside	Acceptable
BSC Class II-A1	Product, personnel, and environment	Inward (75)	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 positive pressure.	Minute amounts only if thimble connected to exhaust ¹
BSC Class II-A2	Product, personnel, and environment	Inward (100)	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 negative pressure.	Minute amounts only if thimble connected to exhaust ¹
BSC Class II-B1	Product, personnel, and environment	Inward (100)	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	Limited amounts ¹
BSC Class II-B2	Product, personnel, and environment	Inward (100)	Exhaust connection must be hard ducted to the outside. 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.	Acceptable
BSC Class III, Isolator, Glove box	Maximum Product personnel, and environmental	Inward	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, superclean manufacturing without creating clean room, pharmaceutical manufacturing of potent compounds, BL4 agents).	Limited amounts ¹

¹ In no circumstances should the chemical concentration approach the lower explosion limits of the compound.

Sources: Adapted from NSF Standard 49 and Primary Containment for Biohazards; Selection, Installation and Use of Biological Safety Cabinets, Current Edition. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health

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.

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 nonsterile 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 3 minutes before commencing procedures. Never use the view screen above the 8-inch mark.
- ✓ 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; 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 safer equipment, such as a burner with a pilot light that provides a flame only on demand or a flameless alternative.
- ✓ Place contaminated items such as pipettes in a disinfectant-filled pan 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 bag. 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 30 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 BL2 and BL3.** For more information about this service, contact the Environmental Health Program.

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 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 Safety Department Web site and upon request. Consult the *UW Laboratory Safety Guide* for instructions.

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, and body fluids from humans
- Tissues, liquid blood, 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 and exotic or virulent plant and animal pathogens. 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 to assure custodial/waste disposal personnel that containers are safe to handle.

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 to prevent wounding of coworkers, custodial personnel, 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 disposal. Medical sharps need not be autoclaved prior to disposal by MERI unless they come from a BL3 facility. If you plan to autoclave the sharps container, make sure it is made from heat resistant material.

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, nonfragile 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 of 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. 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 *Geobacillus 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.

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 nonlipid 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 Safety 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 0.05–0.5%. Solutions should be

stored in an opaque bottle to reduce decay during storage. A fresh 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.

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 Safety Department for disposal by this method. Bedding, plastic, and metallic objects must be excluded from packages of animal carcasses. Consult the Safety 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 those 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. 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:

- 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 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 UW Police for transportation to the University Hospital emergency room at any hour.
- 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. Currently, there is no known biohazard on the UW campus that would prohibit properly garbed and masked fire or security personnel from entering any biological laboratory in an emergency.

Reporting is an additional required step in emergency management. The supervisor should always be notified and an accident report 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, seek immediate medical assessment from either University Hospital Employee Health Service (Monday through Friday, 7:30 a.m. to 4:30 p.m., 263-7535) or the University Hospital Emergency Department (all other times, 262-2398). Employee Health Service is located at University Station, 2820 University Avenue.

Investigators 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 to describe briefly their recommended 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*.

Transport of Dangerous Goods

The transportation of dangerous goods is regulated by U.S. Department of Transportation. The employer must certify training and the training must cover function specific aspects, safety, security, and general awareness. The requirements for proper packaging, labeling, marking and declaration apply fully when a commercial carrier provides service to transport the hazardous materials.

There are times when UW-Madison employees transport biological materials, some of which meet the regulatory definition of a dangerous good, between buildings on the main campus or to outlying areas in an institutional vehicle. It may be impractical to hire a commercial carrier and it can be done safely with advance planning. This activity does not meet the regulatory definition of transportation in commerce and is allowed under the

regulations. The regulatory standard of care still applies and you should follow virtually all of the procedures that you would use when employing a commercial carrier. You would not need a dangerous goods declaration, nor would you use the institution's contract for emergency response information.

The following steps should be used when transporting infectious substances without involving a commercial carrier:

- 1) Use UN certified packaging that is appropriately marked and labeled.
- 2) Prepare an MSDS-equivalent for the pathogen that will accompany the shipment.
- 3) Train the worker about nature of the pathogen and emergency procedures.
- 4) Have an emergency response kit (e.g., absorbent, disinfectant, biohazard bags) available.

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.

The degree to which laboratory security is implemented should be commensurate with risk. All laboratories, including those handling only low-risk biological materials under BL1 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 BL1 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

Roles and Responsibilities

Office of Biological Safety

The Office of Biological Safety (OBS) fosters safe laboratory practices and ensures compliance with and 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 the following services:

- 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) and infectious waste.
- 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.
- Maintains a library of safety literature.
- Provides biohazard signs.
- Provides training and certification for compliance with U.S. Department of Transportation regulations for shipping hazardous biological materials.

Institutional Biosafety Committee

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 (also known as the Biological Safety Committee) 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 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 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 during the academic year) to review rDNA research and investigations that involve biohazardous materials. To facilitate the review process, investigators should submit their biosafety protocol to the OBS with sufficient lead time, minimally two weeks before the meeting date. Materials received less than one week (five working days) before a scheduled IBC meeting will not be considered until the following meeting.

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; toxins; experimental, biologically active chemicals (carcinogens, mutagens, and teratogens); human blood, body fluids, and tissues; 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, 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 use of safe work practices and for following safety-related instructions from supervisors. Principal Investigators, instructors, and laboratory supervisors have a special obligation 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 Web site.

Risk assessments of planned experiments should be done prior to initiation. The investigator'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 preinitiation 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 Web site. 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, the U.S. Department of Agriculture, 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 be notified of intended field studies of genetically engineered organisms.

Wisconsin Department of Natural Resources Guidelines for Waste Disposal: The DNR has established regulations (*Medical Waste*, Oct. 1994) for the decontamination and elimination of infectious and medical wastes. Appropriate disposal of these wastes is an important aspect of a comprehensive safety program.

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).

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; provide a copy of any required federal permit.
- Exotic plants, animals, and microbes (e.g., nonindigenous plants or insect pathogens, or biological control agents); provide a copy of any required federal permit.
- 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 Web site 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*.

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 Web site 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 Web site 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 and animal care protocols 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, this procedure 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.

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 two weeks prior to an IBC meeting, which tentatively is scheduled for the first Wednesday of each month. Materials received less than one week (five working days) before a scheduled IBC meeting will not be considered until the following meeting. The schedule is posted at the OBS Web site.

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.

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, not a compliance audit.

Training

Biosafety

Biological safety training is offered by OBS in several formats. The Basic Biosafety class and specialized classes are presented periodically and upon request. The content of the Basic Biosafety class also is available as a Web-based session. Web-based training on use of the biological safety cabinet (BSC) is also available (see below).

Laboratory surveys for BL1 through BL3 are provided at the OBS Web site. These checklists, which are based on CDC and NIH recommendations, provide a methodical tool to review appropriate practices and also can serve as a training instrument.

A log of training should be maintained, including not just formal classroom sessions but also topics covered during staff meetings and one-on-one mentoring.

Proper Biological Safety Cabinet (BSC) Selection, Use, and Maintenance

Computer-based training is available for UW-Madison faculty, staff, and students at the OBS Web site. The training is divided into five units:

Unit 1: Describes different primary ventilation equipment (fume hood, clean air device, BSC) and the types of protection each provides.

Unit 2: Describes how BSCs work: airflow patterns, protection, HEPA filters, and exhaust connections.

Unit 3: Describes types of BSCs: Class I, Class II (Types A1, A2, B1, B2), and Class III. These unique classes of cabinets offer different types and levels of protection.

Unit 4: Describes how to use a BSC effectively: locating it in your lab, preparing to work, working in the BSC, and cleaning the BSC.

Unit 5: Describes the testing a BSC must undergo to ensure that it is providing personal, product, and environmental protection.

Print out and use the instructions to go through the training. Quizzes are included to test your understanding and must be completed before you pass to the next unit. To document your training, print out and retain the results of the submitted quizzes.

Shipping Hazardous Biological Materials

Hazardous materials, capable of posing an unreasonable risk to health, safety, and property, are commonplace in university facilities. Among them are chemicals and solvents, cleaning agents, radionuclides, infectious agents, and toxins. 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 requires all persons involved in shipping hazardous materials to be trained and certified in proper handling of these materials. Activities for which training is required include

- preparing shipping papers;
- loading and unloading trucks;
- marking and labeling packages;
- filling packages;
- supervising these activities.

The Safety Department offers training to address this requirement. The OBS provides training according to the Dangerous Goods Regulations of the International Air Transport Association, with a focus on shipping infectious substances and other biological materials, as well as the cryogenic materials and chemicals that may accompany biological samples. Certification is valid for two years under these stringent air transport requirements. Renewal may be done by attending class or by completing a Web-based training session.

BioSide Lines

Newsletter of the Office of Biological Safety

The OBS issues a quarterly newsletter that supplements the guidance provided in this biosafety manual. It covers topics of current interest regarding laboratory safety and regulatory compliance. The newsletter is distributed electronically to Principal Investigators who have active biosafety protocols and to personnel who have completed the biosafety and hazardous materials shipping training. The current and previous issues are available at the OBS Web site.

Useful References

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

American Biological Safety Association list of risk groups, available on request from OBS.

Arthropod Containment Guidelines. Version 3.1 (12/01) A project of the American Committee of Medical entomology of the American Society of Tropical Medicine and Hygiene. **<http://www.astmh.org/subgroup/archive/ACGv31.pdf>**

Biosafety in Microbiological and Biomedical Laboratories, CDC/NIH. 1999 4th edition.

<http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.ht>

[Note: A revision is anticipated to be released in 2005.]

Health Canada, Biological Safety Data Sheets

<http://www.hc-sc.gc.ca/pphb-dgspsp/msds-ftss/index.html>

National Sanitation Foundation Standard (NSF) 49, Biological Safety Cabinets, 2002

NIH Guidelines for Research Involving Recombinant DNA Molecules.

<http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html>

NTP Report on Carcinogens. National Toxicology Program, Department of Health and Human Services. **<http://ehp.niehs.nih.gov/roc/toc10.html>**

[Note: 11th edition to be released January 2005.]

OSHA Lab Standard. Occupational exposure to hazardous chemicals in laboratories.

29 CFR 1910.1450 Appendix A - National Research Council Recommendations Concerning Chemical Hygiene in Laboratories (Non-Mandatory).

Public Health Service, U.S. Department of Health and Human Services, CDC/NIH.

Primary Containment for Biohazards: Selection, Installation, and Use of Biological Safety

Cabinets, 2nd edition, September 2000. **<http://www.cdc.gov/od/ohs/biosfty/bsc/bsc.htm>**

TOXNET, a cluster of databases on toxicology, hazardous chemicals, and related areas.

The National Library of Medicine. **<http://toxnet.nlm.nih.gov/>**

Traynor et al. 2001. A Practical Guide to Containment: Greenhouse Research with Transgenic Plants and Microbes. Information Systems for Biotechnology.

www.isb.vt.edu/cfdocs/greenhouse_manual.cfm

UW-Madison Safety Department, Chemical and Radiation Protection Office,

UW Laboratory Safety Guide. **www.fpm.wisc.edu/chemsafety/Guide/toc.htm**

World Health Organization Laboratory Biosafety Manual. 2nd edition, revised.

Geneva, 2003. **www.who.int/csr/resources/publications/biosafety/Labbiosafety.pdf**

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

Archaeobacterium 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, *Cl. chauvoei*, *Cl. haemolyticum*, *Cl. histolyticum*, *Cl. novyi*,
Cl. septicum, *Cl. 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. bovis* BCG vaccine strain, *M. chelonae*, *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. choleraesuis*, *S. enteritidis*, *S. gallinarum-pullorum*, *S. meleagridis*, *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. (Xylohypha) trichoides*
Cryptococcus neoformans
Dactylaria galopava (Ochroconis gallopavum)
Epidermophyton

Exophiala (Wangiella) dermatitidis
Fonsecaea pedrosoi
Microsporium
Paracoccidioides braziliensis
Penicillium marneffei
Sporothrix 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. granulosus*, *E. multilocularis*, *E. vogeli*
Entamoeba histolytica
Enterobius
Fasciola including *F. gigantica*, *F. hepatica*
Giardia including *G. lamblia*
Heterophyes
Hymenolepis including *H. diminuta*, *H. nana*
Isospora
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. sui 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

Rickettsia akari, *R. australis*, *R. canada*, *R. conorii*, *R. prowazekii*, *R. rickettsii*,
R. siberica, *R. tsutsugamushi*, *R. typhi* (*R. mooseri*)

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
 - Hantaviruses 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 (TME) 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

Group 4 (RG4) - Fungal Agents

None

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

Flaviviruses (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 BL1 containment.

Appendix B

BL3 Manual: Points to Consider

The purpose of preparing a BL3 manual is to provide detailed procedures that staff follow while working in your facility under BL3 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*, 4th ed., Section III
<http://www.cdc.gov/od/ohs/biosfty/bmb14/bmb14s3.htm>

NIH *Guidelines for Research Involving Recombinant DNA Molecules*, Appendix G-II-C
http://www4.od.nih.gov/oba/rac/guidelines_02/Appendix_G.htm

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 plasticware 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 needlestick?

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?

Notes

Contact Information

Biohazard information
General biosafety information
Protocol registration
Recombinant DNA guidelines
Shipping and permits for biological materials
Laboratory ventilation (HVAC)
Laboratory containment design

Office of Biological Safety
(Safety Department)
263-2037

Hazardous waste disposal
(chemical, radioactive, animal carcasses)
Chemical hygiene plans
Radioactive materials

Chemical and Radiation Protection Office
(Safety Department)
265-5518

Animal contact health information
Workplace exposures
Bloodborne pathogens
Zoonosis issues
Medical clearances for respirators

Occupational Health Office
(Safety Department)
263-2177

Biological safety cabinet
certification and repair

Environmental Health Program
262-1809

Research animal use and care
Animal care protocols

Research Animal Resources Center
262-1238

Poison information

Regional Poison Control Center
262-3702

Police, fire, personal injury, crime

Emergency 911
Nonemergency 262-2957

Employee Health Service
Emergency Department

UW Hospital and Clinics
263-7535
262-2398