The UW-Madison Researchers' Biosafety Manual

UW-Madison Institutional Biosafety Committee and Office of Biological Safety Policies, Requirements, and Recommendations Manual

Institutional Biosafety Committee (IBC)
Office of Biological Safety (OBS)
University of Wisconsin–Madison
http://www.ehs.wisc.edu/biosafety

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INSTITUTIONAL BIOSAFETY COMMITTEE CHARGE

The Institutional Biosafety Committee (IBC) at UW-Madison originated in 1972 when Chancellor Edwin Young established the Biological Safety Committee. The stated purpose of the committee was to address general concerns about the "increased use in research of biological materials that may result in inadvertent exposure of laboratory personnel and the general public to potentially dangerous infectious agents which either occur naturally or are the result of laboratory manipulations…" The goal of the committee was to identify potential hazards and to assure that adequate precautions are taken. In 1979, Chancellor Irving Shain delegated further authority to the committee to assure compliance with the *NIH Guidelines for Activities Involving Recombinant DNA Molecules* and to promote biological safety for all activities which involve the use of biological materials.

The current charge of the committee was issued in 2015 by Chancellor Rebecca Blank. Chancellor Blank clarified that the IBC is charged with responsibility for oversight of research using biological materials that entails a potential risk to humans, animals, plants, or the environment. This research includes, but is not necessarily limited to, studies involving recombinant DNA, infectious agents, toxic chemicals used to elicit a biological response, or other substances that may be toxic to living organisms. The IBC is authorized to approve, require modifications to secure approval, or disapprove these proposed research activities. The IBC is further authorized to suspend or revoke authorization for previously approved research that is not being conducted in accordance with the approved protocol, the IBC's requirements, federal or state laws or regulations, or institutional policies applicable to biological research. The IBC may also suspend or revoke authorization for previously approved research when the research or its conduct creates an unexpected serious potential threat to safety, health, or the environment. In addition, the IBC is authorized to draft and implement policy and to set other requirements related to the use of biological materials in research or teaching, and to conduct assessments of potential Dual Use Research of Concern.

The Office of Biosafety (OBS) provides biosafety guidance to the UW-Madison research community and serves the IBC in an administrative role. OBS promulgates biological aspects of safety through laboratory visits, consultations, and training as well as following regulations regarding biosafety in laboratory spaces.

The IBC is mandated by the U.S Department of Health and Human Services, National Institutes of Health (NIH) - *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (**https://osp.od.nih.gov/wp-content/uploads/2019_NIH_Guidelines.htm**,)The responsibilities of this committee extend beyond recombinant DNA activities to all biohazardous materials. In addition to the Guidelines, the recommendations of the U.S. Department of Health and Human Services, Centers for Disease Control and Prevention (CDC) and National Institutes of Health (NIH) - Biosafety in Microbiological and Biomedical Laboratories (BMBL), current edition, (**http://www.cdc.gov/biosafety/publications/index.htm**) are adopted as standards of conduct.

IBC POLICIES

Policies and Procedures Adopted by the IBC

Note: This section will be updated as the IBC continues to approve policies
The IBC sets the date effective on a case-by case basis for each policy.

Title/Link	Number
Institutional Biosafety Committee (IBC) Conflict of Interest Policy	UW-6087
Institutional Biosafety Committee (IBC) Policy for Animal Waste and Carcass Disposal	UW-6090
Institutional Biosafety Committee (IBC) Policy for Compulsory Biosafety Training	UW-6080
Institutional Biosafety Committee (IBC) Policy for Suspension of Previously Approved Research	UW-6085
Institutional Biosafety Committee (IBC) Policy on Dengue Virus and Laboratory Information Acknowledgment Form	UW-6084
Institutional Biosafety Committee (IBC) Policy on Reporting Laboratory-Acquired Infections to State and Local Public Health Authorities	UW-6082
Institutional Biosafety Committee (IBC) Policy on Vaccinia Vaccination and Waiver Form	UW-6083
Institutional Biosafety Committee (IBC) Policy for Expired Biosafety Protocols	UW-6092
Institutional Biosafety Committee (IBC) Policy for Principal Investigator on A Biosafety Protocol	UW-6081
Institutional Biosafety Committee (IBC) Policy for Reporting Biosafety Concerns	UW-6091
Institutional Biosafety Committee (IBC) Policy on Review of Research Protocols	UW-6076
Institutional Biosafety Committee (IBC) Policy on Access to IBC Meeting Minutes and Other Records	UW-6072
Institutional Biosafety Committee (IBC) Policy on Appropriate Containment for Select Opportunistic and Borderline Pathogens	UW-6089

Institutional Biosafety Committee (IBC) Policy on Biosafety Cabinet (BSC), Animal Transfer Stations (ATS), and Clean Air Devices (CAD)	UW-6088
Institutional Biosafety Committee (IBC) Policy on Conduct of Meetings in Open Session	UW-6071
Institutional Biosafety Committee (IBC) Policy on Core Facility Registration Responsibilities	UW-6077
Institutional Biosafety Committee (IBC) Policy on Maintenance of Ventilated Cage Racks	UW-6100
Institutional Biosafety Committee (IBC) Policy on Principal Investigator Requests for Reconsideration Process	UW-6086
Institutional Biosafety Committee (IBC) Policy on Receipt and Transmission of Public Comments	UW-6073
Institutional Biosafety Committee (IBC) Policy on Reporting Exposures, Injuries, Releases and Other Incidents	UW-6075
Institutional Biosafety Committee (IBC) Policy on Shared Use of Laboratory or Animal Facilities	UW-6078
Institutional Biosafety Committee (IBC) Policy on UW-Madison IBC Member Training	UW-6079
Institutional Biosafety Committee (IBC) Policy on the Registration of Transgenic Animals	UW-6074
DURC Decision Appeal Process Policy	UW-6097
DURC Development and Review of Risk Mitigation Plans Policy	UW-6099
DURC Education and Training Policy	UW-6098
DURC ICDUR Responsibilities Policy	UW-6094
DURC Principle Investigator Responsibilities Policy	UW-6093
DURC Review Process and Risk Assessment Policy	UW-6096
DURC Subcommittee Policy	UW-6095
Bloodborne Pathogen Exposure Program for Research Areas	UW-6103
Minors in Research Laboratories Policy	UW-6016
UW-Madison Select Agent Program	Info-111

PRINCIPAL INVESTIGATOR (PI) RESPONSIBILITIES

Biological Safety Protocol

OBS and the IBC monitor and review research through the use of a Biological Safety Protocol. The PI is responsible for maintaining an up to date biological safety protocol, if their research at UW-Madison involves any of the following:

- Recombinant (transgenic) or synthetic DNA/RNA organisms or materials, including human gene therapy
- Microbes and disease-causing agents including bacteria, viruses, fungi, prions, protozoa, and parasites
- Large scale propagation consisting of a volume greater than 10L or more in one vessel
- Human cells and cell culture, organs or tissues, or biological samples
- Non-human cells and cell culture, organ or tissues, or biological samples that are infectious, potentially infectious, or recombinant

- Animals (vertebrate and/or invertebrate) that are recombinant (transgenic), exotic, and/or grown in association with pathogens, biological toxins, and/or recombinant materials
- Plants that are recombinant (transgenic), exotic, and/or grown in association with biological toxins, pathogenic or recombinant microbes and/or pathogenic or recombinant small animals (insects, etc.)
- Biological Toxins (this does not include toxic chemicals or antibiotics)

Training

All faculty, staff, and students working with biohazardous and/or recombinant materials must complete the Biosafety Required Training course. In addition, laboratory specific training should be provided for all staff and students.

Principal Investigator Assurance Statement

The Principal Investigator (PI) is responsible for the scientific research within a laboratory, training of laboratory personnel, and must abide by and adhere to all UW-Madison IBC policies, *NIH Guidelines*, *Biosafety in Microbiological and Biomedical Laboratories*, and any other applicable regulations. The PI must understand, adhere to and sign the UW-Madison IBC assurance statement contained in their biosafety protocol and included below.

Assurance Statement

I certify that the information contained in this application is accurate and complete. I am familiar with and agree to abide by *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecule*s, *current ed.* and *Biosafety in Microbiological and Biomedical Laboratories, current ed.*

Also, I agree to abide by the following requirements:

a. I will not initiate any biological research subject to the guidance and guidelines mentioned above until that research has been registered, reviewed, and approved by the UW-Madison Institutional Biosafety Committee (IBC). The purview of the UW-Madison IBC includes biological research involving recombinant nucleic acids; biological agents and pathogens; human cells, tissues, materials and embryonic stem cells; select agents and toxins, biological toxins, synthetic nucleic acids and the use of any of these in animal or plant research.

b. I will assure that personnel, including animal care staff or other laboratory support staff, have received appropriate information including signage, about the biological hazards of the research outlined in this registration by making available copies of approved protocols, Biosafety Manuals, and Biological Research Registrations that describe the potential biohazards and precautions to be taken to prevent exposures or release to the laboratory or the environment.

c. I will ensure that laboratory personnel understand the procedures for dealing with incidents and spills of biological materials and know the appropriate waste management procedures.

d. I will comply with all training and shipping requirements for the transport of hazardous biological materials according to the US Dept. of Transportation (DOT) 49 CFR 171-178, International Civil Aviation Organization (ICAO) and International Air Transport Association (IATA).

e. I will comply with the OSHA/DOC Bloodborne Pathogen Standard 29 CRF 1910.1030 if my research includes human cells, tissues, materials or embryonic stem cells.

f. I will ensure that all laboratory personnel working with biological materials are listed on this registration.

g. I will assure that I along with all laboratory personnel have completed all required biosafety training and that their training records are up to date.

h. I assure that all laboratory spaces associated with the research described in this registration are listed.

i. I am familiar with and understand my responsibilities as a Principal Investigator as outlined in Section IV-B-7 of the *NIH Guidelines.*

j. I will assure adequate supervision of personnel and will correct work errors and conditions that could result in breaches of the guidelines and regulations pertaining to this research as listed above.

k. I will immediately inform the UW-Madison Office of Biological Safety of any spills outside containment, potential exposures or breaches of the guidelines and regulations listed above and will submit the *First Report of Biological Exposure or Release Form* within 24 hours.

Principal Investigator: _____

Signature:_____Date:_____

GENERAL PRINCIPLES OF BIOLOGICAL SAFETY

Risk Assessment

Risk assessment is the process that enables the appropriate selection of laboratory practices, safety equipment, and facility safeguards for handling biohazardous materials such as known infectious or potentially infectious agents or materials. The Office of Biological Safety considers the following when evaluating a known infectious or potentially infectious agent or material:

- The agent's biological and physical nature
- The concentration and suspension volume of the agent
- The sources likely to harbor the agent
- Host susceptibility
- The procedures that may disseminate the agent
- The best method to effectively inactivate the agent

Biohazard Containment

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

Practices and Procedures

Standard microbiological practices are common to all laboratories. Special microbiological practices, safety equipment, and laboratory facilities enhance worker safety, environmental protection, and minimize the risk of handling agents. Please see the CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL) website at http://www.cdc.gov/biosafety/publications/index.htm (Section IV) regarding information on Laboratory Biosafety Level criteria, etc. It is the responsibility of all laboratory staff to effectively decontaminate equipment before it is removed from the laboratory for maintenance, relocation, sale, or disposal.

Biosafety Level 1

Biosafety Level 1 (BSL1) is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel, animals, or the environment. BSL1 laboratories are not necessarily separated from the general traffic patterns in the building. Work can typically be conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science.

Biosafety Level 2

Biosafety Level 2 (BSL2) builds upon BSL1. BSL2 is suitable for work involving agents that pose moderate hazards to personnel, animals, or the environment. It differs from BSL1 in that: 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

Biosafety Level 3

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause

serious or potentially lethal disease through the inhalation route of exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents and must be supervised by scientists competent in handling infectious agents and associated procedures. All procedures involving the manipulation of infectious materials must be conducted within BSCs or other physical containment devices. A BSL3 laboratory has special engineering and design features.

BSL3 Manual Requirements

A BSL3 Manual is needed for all BSL3/ABSL3/ACL3 laboratories. The manual provides a summary of procedures and practices for staff to follow while working in the BSL3 laboratory. The manual is required to be specific to your laboratory, facility, agents, and procedures used, and is reviewed as part of the risk assessment. It is required that the manual and the biosafety protocol be reviewed annually by the laboratory and updated to reflect any changes in the laboratory that may affect the risk assessment.

At minimum the elements below must be addressed in a BSL3 manual. Some of the elements may not be applicable to your laboratory and are *italicized* in the list below:

1. Title, table of contents
2. Emergency Contacts
3. Revision history and Record Keeping
    a. Specific changes made, who made the changes and date
    b. Review dates (e.g., IBC review and annual review)
4. Facility Design and Specifications
    a. Number and location of rooms
    b. Engineering controls (e.g., HEPA filters, exhaust, air handling systems, pressure gauges)
    c. Generator for power outages
    d. Security description (e.g., fingerprint scanners, retina scanners, ID card scanners, high security keys)
    e. Waste collection system (e.g., Effluent Decontamination System (EDS))
5. Facility Reverification
    a. Annual certifications or performance testing (e.g., BSC, Fume Hood, HVAC, HEPA filters)
    b. Description of procedures performed during reverification (e.g., preventative maintenance of equipment, work stopped, materials secured, biohazard trash removed, decontamination of surfaces performed)
    c. Describe record keeping (e.g., EH&S keeps records of facility reverification)
6. Facility Decontamination
    a. Yearly requirements
    b. At the end of facility use
    c. When repairs are needed
7. Biosafety Level 3 (BSL3) Description: (BMBL, these elements may be covered in other sections)
    a. Standard Microbiological Practices

   b. Special practices (e.g., describe special regulations for non-research related plants, animals)
   c. Safety Equipment
   d. Laboratory Facilities
8. *Animal Biosafety Level 3 (ABSL3) Description: (BMBL, these elements may be covered in other sections)*
   a. Standard Microbiological Practices
   b. Special practices
   c. Safety Equipment
   d. Laboratory Facilities
9. Personnel Requirements
   a. Training
      i. Describe training procedures
      ii. Documentation of personnel proficiency
      iii. Documentation of training/notification of BSL3 manual/research activity changes
   b. Any applicable background checks, clearances, evaluations
   c. Understanding of hazards
      i. Microbes
         1. List agent(s) and describe brief history, host range, route of transmission, biosafety level practices recommended, genetic manipulation performed on the agent(s) (how that affects the risk assessment), other important information to understand the risk of manipulating the agent(s)
         2. *May also develop a laboratory-specific medical response sheet*
         3. *May also develop a pathogen specific data sheet*
      ii. Other biological hazards (e.g., toxins, cell lines, etc.)
      iii. Procedural hazards
      iv. Other hazards
   d. Understanding health and medical requirements
   e. Describe fit testing of respirators (testing, medical clearance, refer to detailed SOP for respirator use)
   f. Understand and comply with any agent specific programs (quarantine, allergy etc.)
10. Service Personnel and Visitor Requirements
   a. Escorting
   b. Use of PPE
   c. Visitor training (e.g., hazard communication)
   d. Visitor log
   e. Vaccination requirements

11. Health and Medical Monitoring
   a. Depending on the agent, certain restrictions, vaccinations or monitoring may need to be in place for BSL3 work.

   b. Outline as applicable to your agent (e.g., symptoms for each agent, reporting, what to do when you are sick, emergency procedures, contact numbers, testing requirements)
   c. List types of accidental exposure:
      i. Needle stick
      ii. Animal Bite
      iii. Break in PPE
      iv. Broken vessel outside BSC
      v. Unknown exposure with symptoms
12. Laboratory Research Practices
   a. List specific practices beyond the already required inherent BSL1 and BSL2 practices required to work at BSL3
   b. Describe any special practices for: specific agents (e.g., agent A may not be handled when agent B is being handled); activities performed outside of containment (e.g., use of specialized equipment)
   c. Cleaning and maintaining equipment and surfaces (e.g., frequency, disinfectant exposure time and concentration, eye wash maintenance)
   d. Describe animal experimental procedures or summarize and reference specific SOPs. (cleaning, housing, monitoring, PPE, who performs tasks)
   e. Procedures for waste removal
   f. Record keeping
13. Decontamination of Laboratory Waste
   a. Autoclave use
   b. Documentation
   c. Efficacy testing
   d. Equipment decontamination (large and small) procedures
   e. Animal waste decontamination (cages, waste, bedding, animals)
   f. Sharps disposal
14. Emergency Response
   a. Detail spill protocols (inside and outside of containment)
   b. List emergency contacts and reporting procedures
   c. Exposure procedures
   d. Breech of containment
   e. Health Emergency/Fire/Weather Emergency
   f. Theft/missing agents
15. Removal of equipment from the BSL3 area (e.g., maintenance, repair, replacement)
   a. Detail decontamination procedures and documentation
16. Material Intake and Removal Procedures
   a. Shipping and receiving requirements/training
   b. Documentation (inactivation, confirmation, validations, assurances)
   c. Permit requirements
17. Pest Control
   a. Insect and Pest Control Program written and in place
   b. Describe regulation requirements
18. Appendices:
   a. Relevant SOPs

b. Facility map
c. Consent forms
d. Training forms
e. List of approved users
f. Entry and exit
g. PPE donning and doffing
h. Inactivation of infectious materials
i. Infectious waste disposal
j. Decontamination of BSC and items in BSC
k. Trash disposal
l. Record keeping
m. Additional information as needed or per IBC
n. Chart on risk assessment for specific activities (e.g., deviation from typical BMBL practices)

Table 1. Summary of Safety Practices for Biosafety Levels 1-3

This information was compiled from BMBL, UW Madison IBC Policy, and Biosafety Principles and Practices. During the review of biosafety protocols, risk assessment will determine the required containment and practices for specific research activities.

	BSL 1	BSL 2	BSL 3
Before Entering			
Access to the laboratory is controlled.	Yes	Yes	Yes
Doors are kept closed when not entering or exiting.	Yes	Yes	Yes
Laboratory Emergency Information door card must be posted at the entrance to the laboratory. Reviewed and dated annually for accuracy.	Yes	Yes	Yes
A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory. The sign includes the biohazards in use and the name and phone number of the laboratory supervisor or other responsible personnel. Reviewed and dated annually for accuracy.	No	Yes	Yes
Hazards communication-all persons, including visitors and service personnel, entering the lab must be advised of the potential hazards and meet specific entry/exit requirements, such as vaccinations, TB testing or donning and doffing of PPE.	Desirable	Yes	Yes
The laboratory personnel must receive lab specific training regarding hazards, risk mitigation, emergency procedures, and occupational health considerations. Training must be documented. The IBC recommends refresher training at least once per year.	Yes	Yes	Yes
Laboratory personnel must be provided medical surveillance, as appropriate, and offered available	No	Yes	Yes

immunizations for agents handled or potentially present in the laboratory.			
The laboratory supervisor, Principal Investigator, or designee must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological and/or laboratory techniques/practices before working with all materials/agents.	Desirable	Yes	Yes
Animals and plants not associated with the work being performed must not be permitted in the laboratory.	Desirable	Yes	Yes
Practices to reduce cardboard are in place.	Desirable	Desirable	Yes
Practices to reduce pests, mold, fire hazards are in place (e.g., reduce cardboard, reduce clutter).	Desirable	Desirable	Yes
All personnel will don minimal entry PPE as listed in the biosafety protocol prior to entry.	Yes	Yes	Yes
Within Laboratory			
Don extra PPE according to activities being performed.	Yes	Yes	Yes
Mouth pipetting is prohibited; pipetting devices must be used.	Yes	Yes	Yes
Procedures for safe handling of medical sharps (e.g., needles, scalpels) and non-medical sharps (e.g., pipettes, broken glassware) must be followed.	Yes	Yes	Yes
Plastic ware should be substituted for glass wherever possible.	Desirable	Desirable	Yes

Potentially infectious materials must be placed in a durable, leak-proof container during collection, handling, processing, storage, or transport within a facility. If this is not possible, a risk assessment is performed.	Yes	Yes	Yes
Perform procedures to minimize the creation of splashes and/or aerosols.	Yes	Yes	Yes
All procedures involving the manipulation of infectious materials that may generate an aerosol are conducted within a BSC or other physical containment device.	Desirable	Desirable	Yes
Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.	Yes	Yes	Yes
Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.	Yes	Yes	Yes
All cultures, stocks, and other forms of potentially infectious or recombinant materials must be inactivated using an effective method either by the laboratory or an approved vendor before disposal.	Yes	Yes	Yes
Spills involving infectious materials must be contained, decontaminated, and cleaned up by laboratory personnel properly trained and equipped to work with infectious material.	Yes	Yes	Yes
Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to	Yes	Yes	Yes

procedures described in the laboratory biosafety protocol.			
Decontaminate work surfaces after completion of work and after any spill or splash of potentially hazardous material with an effective disinfection method.	Yes	Yes	Yes
Materials and People Leaving the Laboratory			
Persons must wash hands before leaving the laboratory.	Yes	Yes	Yes
Practices to prevent spills and potential exposures during movement of materials in hallways, elevators, or public spaces are in place.	Yes	Yes	Yes
Transport of biohazardous materials should occur in a secondary container that is durable, leak proof, labeled, and surface disinfected.	Yes	Yes	Yes
Biowaste should be transported in a manner that prevents leaks, spills, exposures, or releases.	Yes	Yes	Yes

Vertebrate Animal Biosafety Level Criteria for Vivarium Research Facilities

Please also see the CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL) website at http://www.cdc.gov/biosafety/publications/index.htm (Section V) regarding information on Vertebrate Animal Biosafety Level Criteria.

This guidance is provided for the use of experimentally infected animals housed in indoor research facilities (e.g., vivaria), and is also useful in the maintenance of laboratory animals that may naturally harbor zoonotic infectious agents. In both instances, the institutional management must provide facilities, staff, and established practices that reasonably ensure appropriate levels of environmental quality, safety, security and care for the laboratory animal. Laboratory animal facilities are a special type of laboratory. As a general principle, the biosafety level (facilities, practices, and operational requirements) recommended for working with infectious agents *in vivo* and *in vitro* are comparable.

The animal room can present unique problems. In the animal room, the activities of the animals themselves can present unique hazards not found in standard microbiological laboratories. Animals may generate aerosols, they may bite and scratch, and they may be infected with a zoonotic agent. The co-application of Biosafety Levels and the Animal Biosafety Levels are determined by a protocol-driven risk assessment. These recommendations imply that laboratory animal facilities, operational practices, and quality of animal care meet applicable standards and regulations (e.g., Guide for the Care and Use of Laboratory Animals (https://grants.nih.gov/grants/olaw/Guide-for-the-Care-and-use-of-laboratory-animals.pdf and Animal Welfare Regulations https://naldc.nal.usda.gov/download/5969370/PDF) and that appropriate species have been selected for animal experiments.

In addition, the organization must have an occupational health and safety program that addresses potential hazards associated with the conduct of laboratory animal research. The following publication by the Institute for Laboratory Animal Research (ILAR), Occupational Health and Safety in the Care and Use of Research Animals https://www.nap.edu/catalog/4988/occupational-health-and-safety-in-the-care-and-use-of-research-animals, is most helpful in this regard. Additional safety guidance on working with non-human primates is available in the ILAR publication, Occupational Health and Safety in the Care and Use of Nonhuman Primates. https://www.nap.edu/catalog/10713/occupational-health-and-safety-in-the-care-and-use-of-nonhuman-primates

Facilities for laboratory animals used in studies of infectious or non-infectious disease should be physically separate from other activities such as animal production and quarantine, clinical laboratories, and especially from facilities providing patient care. Traffic flow that will minimize the risk of cross contamination should be incorporated into the facility design. The recommendations detailed below describe four combinations of practices, safety equipment, and facilities for experiments with animals involved in infectious disease research and other studies that may require containment.

In addition to the animal biosafety levels described in this section, the USDA has developed facility parameters and work practices for handling agents of agriculture significance. BMBL Appendix D http://www.cdc.gov/biosafety/publications/index.htm includes a discussion on Animal Biosafety Level 3 Agriculture (BSL-3-Ag). USDA requirements are unique to agriculture because of the necessity to protect the environment from pathogens of economic or environmental impact. BMBL Appendix D also describes some of the enhancements beyond BSL/ABSL3 that may be required by USDA-APHIS when working in the laboratory or vivarium with certain veterinary agents of concern.

Note that the IBC and your IACUC may request an increase in containment to a higher animal biosafety level for some animal experiments. The evaluation of animal studies to determine required biosafety level will include pathogen-specific activities as well as pathogens commonly associated with certain species (rabies virus in bats, for instance).

Animal Biosafety Level 1

Animal Biosafety Level 1 (ABSL1) is suitable for work in animals involving well-characterized agents that are not known to cause disease in immunocompetent adult humans, and present minimal potential hazard to personnel and the environment. ABSL1 facilities should be separated from the general traffic patterns of the building and restricted as appropriate. Special containment equipment or facility design may be required as determined by appropriate risk assessment. (See BMBL Section 2, Biological Risk Assessment (/https://www.cdc.gov/labs/BMBL.html). Personnel must have specific training in animal facility procedures and must be supervised by an individual with adequate knowledge of potential hazards and experimental animal procedures.

Animal Biosafety Level 2

Animal Biosafety Level 2 (ABSL2) builds upon the practices, procedures, containment equipment, and facility requirements of ABSL1. ABSL2 is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment. It also addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure. ABSL2 requires that: 1) access to the animal facility is restricted; 2) personnel must have specific training in animal facility procedures, the handling of infected animals and the manipulation of pathogenic agents; 3) personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations and husbandry procedures; and 4) BSCs or other physical containment equipment are used when procedures involve the manipulation of infectious materials, or where aerosols or splashes may be created. Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment. Implementation of employee occupational health programs should be considered.

Animal Biosafety Level 3

Animal Biosafety Level 3 (ABSL3) involves practices suitable for work with laboratory animals infected with indigenous or exotic agents, agents that present a potential for aerosol transmission, and agents causing serious or potentially lethal disease. ABSL3 builds upon the standard practices, procedures, containment equipment, and facility requirements of ABSL2. The ABSL3 laboratory has special engineering and design features. ABSL3 requires that: 1) access to the animal facility is restricted; 2) personnel must have specific training in animal facility procedures, the handling of infected animals, and the manipulation of potentially lethal agents; 3) personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations, and husbandry procedures; and 4) procedures involving the manipulation of infectious materials, or where aerosols or splashes may be created, must be conducted in BSCs or by use of other physical containment equipment. Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment. Employee occupational health programs must be implemented.

Aquatic Animal Considerations

Aquatic animal research can be very specialized. Distinct considerations are needed for a “wet” facility.

Note: OLAW considers larval and adult forms of fish to be covered by the Public Health Service policy, and both of these forms must be counted. OLAW has made the determination that all stages of zebrafish development greater than 3 days postfertilization (dpf) must be described in animal-use protocols. Zebrafish from 4 days postfertilization onward are considered animals, and therefore must be counted. (Policy: UW-4100 Counting Zebrafish Usage https://policy.wisc.edu/library/UW-4100)

General considerations:

- Water/waste decontaminated prior to release as per local, state, and federal regulations
- Floor and flood drains are considered, and SOPs are in place to prevent releases
- Inspection of facility for areas of water penetration and areas of concern are caulked, sealed or gasketed.
- Risk assessment for any airborne pathogen concerns
- Permit requirements as applicable
- Fish tank waste removal processes
- Disposal and end of study decontamination SOPs
- Specialized hazard communication and/or signage as per risk assessment
- Room surfaces are easy to clean and impervious to moisture (floor, walls, ceiling)
- Training specific to research activities
- Spill protocols:
    - Kits stored away from the floor
    - Similar considerations as for large scale liquid activities

Aquatic facility:

- Location and facility design with respect to potential release into environment
- Release SOPs and reporting SOPs
- Exit and entry points:
    - PPE availability, use
    - Foot baths
    - Signage
    - Emergency information posted (contact information, spill information, etc.)
- Control access (Biosecurity concerns)

Wild-Caught Animal Considerations

Please contact the Office of Biological Safety prior to conducting research with wild-caught animals. Some wildlife species naturally harbor zoonotic infectious agents. In addition, permits may be required from state or federal agencies prior to collecting certain animals from the wild.

Arthropod Biocontainment

Arthropods include, but are not limited to, insects (e.g., mosquitoes, black flies, sand flies, tsetse flies, midges, hemipteran, phthiraptera, siphonaptera, fruit flies, cockroaches, lepidoptera, coleoptera) and arachnids (e.g., ticks, mites, spiders). All life-cycle stages must be considered 'arthropods' (e.g., eggs, larvae, nymphs, adults). Complex life cycles and organism diversity require careful consideration in the risk assessment. Arthropod containment is not specifically addressed in BMBL or the *NIH Guidelines*. Arthropod Containment Guidelines were developed by the American Committee of Medical Entomology as a reference for research involving arthropod vectors of human and animal diseases. In addition to disease-carrying potential, risk assessment for arthropods must consider additional factors such as recombinant modifications, natural range (i.e., is the arthropod an exotic or invasive species in Wisconsin), and ecological role (e.g., is the arthropod considered a pest species). Key elements of the Arthropod Containment are summarized below.

Arthropod Containment Level 1

Arthropod Containment Level 1 (ACL1) is suitable for work with uninfected arthropod vectors or those infected with a non-pathogen including arthropods already present in the local region (regardless of whether there is active vector-borne disease transmission and exotic arthropods that would be non-viable upon escape or only temporarily could establish.

ACL1 practices:

- Arthropod containers and incubators are located to minimize accidental release (e.g., out of the general traffic flow, avoid hallways)
- Materials that provide breeding sites and harborages are minimized to allow detection of escaped arthropods
- Eliminate accidental sources of arthropods (e.g., clean after spills, eliminate standing water)
- Cages or containers are cleaned and maintained to prevent survival or escape
- Bags, rearing trays are used to effectively prevent leakage. Screened mesh may be used if durable and of appropriate size. All life cycle stages are considered.
- All life stages of arthropods are killed prior to disposal (e.g., hot water, freezing)
- Labels attached to container identify species, strain or other relevant information for hazard communication
- Each level of containment and life cycle stage is considered to prevent dispersal on persons
- An effective arthropod trapping program is recommended to monitor the escape prevention program in place
- A program is in place to prevent entrance of wild arthropods and rodents (prevent contamination, predation, inadvertent infection)
- ACL1 signage is posted
- Vertebrate animals used as hosts or blood sources when housed in the insectary, are adequately protected from escaped arthropods
- During feeding on host animals:
    - Primary container integrity examined

    - Precautions in place to prevent escape via flying, screens, covers
    - Host animals are inspected (ears, axillae, fur, etc.) to prevent escape
- Blood sources are considered. If feasible, use of sterile blood or sources known to be pathogen free are used. If human volunteers are used, risk assessment is required.
- Gloves are used when handling host animals
- Lab coats, gowns or uniforms should be worn at all times
- Risk assessment performed to determine the need for arthropod specific PPE (e.g., respirators for allergies, particle masks, head covers)

ACL1 facilities:

- Insectary is separated from general building traffic
- Insectary doors minimize escape and entrance of arthropods (ridged panels, screens, glass, plastic sheets, cloth)
- If facility is not designed as an insectary it may be used considering the following:
    - Arthropods held by a 'cage within a cage'
    - Use of practices preventing escape (e.g., chilling containers before removing mosquitos, non-flying species manipulated in pans within moats of water)
    - Use of Plexiglas glove boxes

Arthropod Containment Level 2

Arthropod Containment Level 2 (ACL2) builds upon ACL1 and is more stringent in physical containment, disposal, and facility requirements. ACL2 is suitable for work with exotic and indigenous arthropods known or suspected to be infected with RG2 agents associated with animal or human disease. It is also appropriate for uninfected genetically modified arthropod vectors of disease provided the genetic modification does not or is not anticipated to increase viability, host range, survival, or vector capacity.

ACL2 practices:

- Laboratory is designed to detect escaped arthropods
    - Equipment and supplies not needed for operation are located outside of the insectary
    - Supplies needed are not located on open shelves (recommend closed storage room or cabinets with tight fitting doors)
    - Insect diet kept in sealed containers
- Cages are shatter proof and screened to prevent escape; may be disposable or autoclavable and designed to prevent escape
- All life stages of uninfected arthropods must be killed prior to disposal freezing or other suitable method
- All life stages of infected arthropods should be autoclaved or decontaminated with chemical disinfectant prior to disposal based on agent specific risk assessment
- Uninfected and infected arthropod containers are clearly labeled; separate rooms are recommended

- Wash hands before leaving the insectary and after handling infected arthropods or cultures
- PPE that is reused is checked for infestation prior to insectary exit
- Species-appropriate escape measure are in place (e.g., oviposition traps, ground-level flea traps, oil-filled channels surrounding tick colonies, light traps for mosquitoes)
- Exterior monitoring for exotic species is recommended
- Breeding and harborage areas are eliminated, furniture and lab space is easy to clean
- Sharps use and disposal as per the biosafety protocol
- Equipment and work surfaces are routinely cleaned as per biosafety protocol
- Appropriate signage is posted
    - BSL2 sign is posted if applicable
    - ACL2 sign is posted (arthropod species, agent, name and phone number of responsible person, any special entry requirements)
- Insectary access is limited to trained persons, guests are escorted and provided hazard communication
- Animals used as hosts or blood sources are not housed with arthropods and protected from escapees
- Living arthropods are moved from ACL1 to ACL2 for infection and are not transported from ACL2 to ACL1
- Escaped arthropods are killed, disposed of or re-captured and returned to their container; infected arthropods are not killed with bare hands and are manipulated with other means
- Accidental release procedure is posted including reporting procedures
- All equipment is decontaminated and disinfested before moving between rooms within the insectary and before removal from the insectary
- Clothing should minimize exposed skin

ACL2 facilities:

- Locate arthropods in dedicated rooms, closets, incubators out of traffic flow to prevent accidental contact and release
    - Recommend at least two self-closing doors; not opened simultaneously
    - Entrance door prevents flying and crawling arthropod escape
    - Increased isolation (e.g., separate building, wing, suite)
    - Windows are not recommended; if present, they need to be resistant to breakage and well-sealed
    - Outlets, vacuum systems, floor drains, plumbing, electrical fixtures are designed to prevent accidental release of arthropods and agents (e.g., filters, traps filled with disinfectant, walls light colored, minimal wall floor and ceiling penetrations, sealed, avoid lighted or dark openings that attract arthropods
    - Additional barriers may be needed based on lab-specific space assessment (e.g., screened partitions, hanging curtains)
    - Recommend dedicated area for handling infected arthropods (e.g., walk-in incubator, screen room, separate cubicle)

- HVAC requirements as per BSL2 laboratory requirements
- Handwashing sinks available

Arthropod Containment Level 3

Arthropod Containment Level 3 (ACL3) containment builds on the ACL1 and ACL2 recommendations and focuses on microbiological containment. ACL3 is suitable for work with arthropods that are known or potential vectors of RG3 microbes or are likely to be infected with RG3 microbes.

ACL3 practices:

- Housing is designed to prevent contact and release of arthropods
    - Incubators serve as an additional layer of containment
    - Less mobile vector arthropods (e.g., ticks) held within vials contained in desiccator cabinets or other escape-proof secondary or tertiary housing if incubator not used
- Manipulation of arthropods in a BSC is difficult and can increase the risks associated with arthropod vector research. Because BMBL states that “All procedures involving the manipulations of infected materials are conducted within a biological safety cabinets or other physical containment devices.” a risk assessment is performed by the IBC for procedures performed outside of containment.
- Materials used to wipe or mop insectary are autoclaved prior to disposal
- Special spill protocols are in place and only trained persons perform spill clean-up
- Special considerations to aerosol generation is needed-even for disinfestation and decontamination of containers. The IBC will review and perform a risk assessment.
- Living infected arthropods should be killed by freezing or other appropriate method prior to autoclaving or incineration
- Only arthropods requiring ACL3 should be housed in the ACL3 insectary
- Viable potentially infected arthropods and infected arthropods of all life stages should not leave the ACL3 insectary. If integral to the research, a risk assessment is performed by the IBC.
- Recommend “count in, count out” method
- Recommend that the number of arthropods in a container be on the label
- Footwear dedicated for the ACL3 is recommended
- Pesticide for emergency use is available in areas of likely arthropod escape
- Operational procedures are reviewed by the IBC

ACL3 facilities:

- Dedicated BSL3 rooms, wings, or suites
- Insectary features:
    - Insectary is locked and access is controlled (e.g., key, proximity reader, or card key)
    - Double door entry with a change room
    - If applicable, showers are plumbed to prevent arthropod escape

- Autoclave available
- Internal doors are self-closing; kept closed during arthropod manipulation
- Additional barriers like hanging curtains are recommended
- Windows are not recommended
- Floor drains are not recommended
- Troughs surrounding door frames may be installed and have a sticky material to trap crawling arthropods, as per risk assessment
- HEPA filtered exhaust may be required for some agents
- HEPA filtered supply recommended for some agents
- Visual directional airflow verification
- Audible alarms present for systems failure
- Engineering testing performed annually

Plant Biocontainment

Biosafety principles are applied to activities involving plants that are exotic, recombinant, and/or grown in association with biological toxins, pathogenic or recombinant microbes and/or pathogenic or recombinant small animals (e.g., insects). The principal purpose of plant containment is to protect the environment, not the researcher. The containment principles are based on the recognition that the organisms that are used pose no health threat to humans or higher animals (unless deliberately modified for that purpose), and that the containment conditions minimize the possibility of an unanticipated deleterious effect on organisms and ecosystems outside of the experimental facility (e.g., the inadvertent spread of a serious pathogen from a greenhouse to a local agricultural crop or the unintentional introduction and establishment of a plant in a new ecosystem). Under special circumstances, which typically require explicit approval from U.S. Department of Agriculture -Animal and Plant Health Inspection Service (USDA-APHIS), it is possible to conduct field trials. Otherwise, release to the environment must be prevented.

Guidance for handling potentially biohazardous plants and associated organisms lags behind that available for vertebrates and their infectious agents. The USDA-APHIS regulates importation, interstate movement, and environmental release of plant pests and transgenic plants but provides minimal guidance for management of facilities. The best available information at this time comes from the *NIH Guidelines.* While the *NIH Guidelines* specifically addresses recombinant DNA, the recommendations regarding effective containment are equally relevant to research using non-recombinant methods.

The information below presents portions of the *NIH Guidelines* that pertain to containment of transgenic plants and associated organisms. The content is consistent with that of the Guidelines but the format has been rearranged to make it more readable. The current version of the *NIH Guidelines* can be accessed at https://osp.od.nih.gov/biotechnology/nih-guidelines/. For detailed information on Plant Biosafety Levels (BSL1-P through BSL4-P), refer to Appendix L of the *NIH Guidelines*.

Containment for transgenic plants and their associated plant pathogens may be achieved by a combination of physical and biological means and relies more heavily on biological factors than is the norm for human and animal infectious agents. The risk

assessment considers the specific organism(s), geographic/ecological setting, and the available mechanical barriers; then the selected practices are tailored to the specific situation. For example, preventing the spread or release of transgenic pollen is a form of biological containment which can be achieved by using sterile lines, altering day length to prevent flowering, or other strategies.

Plant Biosafety Level 1

BSL1-P is designed to provide a moderate level of containment for experiments for which there is convincing biological evidence that precludes the possibility of survival, transfer, or dissemination of recombinant DNA into the environment, or in which there is no recognizable and predictable risk to the environment in the event of accidental release.

BSL1-P relies upon accepted scientific practices for conducting research in most ordinary greenhouse or growth chamber facilities and incorporates accepted procedures for good pest control and horticultural practices. BSL1-P facilities and procedures provide a modified and protected environment for the propagation of plants and microorganisms associated with the plants and a degree of containment that adequately controls the potential for release of biologically viable plants, plant parts, and microorganisms associated with them.

BSL1-P:

- Access to the greenhouse shall be limited or restricted
- Prior to entering the greenhouse, personnel shall be required to read and follow instructions on greenhouse practices and procedures. All procedures shall be performed in accordance with accepted greenhouse practices that are appropriate to the experimental organism.
- A record shall be kept of experiments currently in progress in the greenhouse facility.
- Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.
- A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens)
- Arthropods and other motile macroorganisms shall be housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility.
- Experiments involving other organisms that require a containment level lower than BSL1-P may be conducted in the greenhouse concurrently with experiments that require BSL1-P containment, provided that all work is conducted in accordance with BSL1-P greenhouse practices.
- The term "greenhouse" refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.

- The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas, and is considered part of the confinement area.
- The greenhouse floor may be composed of gravel or other porous material. At a minimum, impervious (e.g., concrete) walkways are recommended.
- Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to contain or exclude pollen, microorganisms, or small flying animals (e.g., arthropods and birds); however, screens are recommended.

Plant Biosafety Level 2

BSL2-P builds upon BSL1-P. BSL2-P is designed to provide a greater level of containment for experiments involving plants and certain associated organisms in 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. Experiments requiring BSL2-P or a higher level of containment involve plants that are noxious weeds, that can interbreed with noxious weeds in the immediate geographic area; or have recognized potential for rapid and widespread dissemination or for serious detrimental impact on managed or natural ecosystems.

BSL2-P:

- A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility.
- If recombinant: The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Greenhouse Director, Institutional Biosafety Committee, NIH OSP and other appropriate authorities immediately (if applicable).
- Experiments involving other organisms that require a containment level lower than BSL2-P may be conducted in the greenhouse concurrently with experiments that require BSL2-P containment provided that all work is conducted in accordance with BSL2-P greenhouse practices.
- A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) the name of the responsible individual, (ii) the plants in use, and (iii) any special requirements for using the area.
- If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence shall be indicated on a sign posted on the greenhouse access doors.
- If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.
- A greenhouse practices manual shall be prepared or adopted. This manual shall: (i) advise personnel of the potential consequences if such practices are not followed, and (ii) outline contingency plans to be implemented in the event of the unintentional release of organisms.
- A greenhouse floor composed of an impervious material. Concrete is recommended, but gravel or other porous material under benches is acceptable unless propagules of experimental organisms are readily disseminated through

soil. Soil beds are acceptable unless propagules of experimental organisms are readily disseminated through soil.
- An autoclave shall be available for the treatment of contaminated greenhouse materials.
- If intake fans are used, measures shall be taken to minimize the ingress of arthropods. Louvers or fans shall be constructed such that they can only be opened when the fan is in operation.
- BSL2-P greenhouse containment requirements may be satisfied by using a growth chamber or growth room within a building provided that the external physical structure limits access and escape of microorganisms and macroorganisms in a manner that satisfies the intent of the foregoing clauses.

Plant Biosafety Level 3

BSL3-P builds upon BSL2-P. BSL3-P describes 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.

BSL3-P:

- Authorized entry into the greenhouse shall be restricted to individuals who are required for program or support purposes.
- Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL3-P practices and procedures. All procedures shall be conducted in accordance with accepted greenhouse practices that are appropriate to the experimental organisms.
- All experimental materials shall be sterilized in an autoclave or rendered biologically inactive by appropriate methods before disposal, except those that are to remain in a viable or intact state for experimental purposes; including water that comes in contact with experimental microorganisms or with material exposed to such microorganisms, and contaminated equipment and supplies.
- Experiments involving organisms that require a containment level lower than BSL3-P may be conducted in the greenhouse concurrently with experiments that require BSL3-P containment provided that all work is conducted in accordance with BSL3-P greenhouse practices.
- Experimental materials that are brought into or removed from the greenhouse facility in a viable or intact state shall be transferred to a non-breakable sealed secondary container. At the time of transfer, if the same plant species, host, or vector are present within the effective dissemination distance of propagules of the experimental organism, the surface of the secondary container shall be decontaminated. Decontamination may be accomplished by passage through a chemical disinfectant or fumigation chamber or by an alternative procedure that has demonstrated effective inactivation of the experimental organism.
- Disposable clothing (e.g., solid front or wrap-around gowns, scrub suits, or other appropriate clothing) shall be worn in the greenhouse if deemed necessary by the Greenhouse Director because of potential dissemination of the experimental microorganisms.

- Protective clothing shall be removed before exiting the greenhouse and decontaminated prior to laundering or disposal.
- Personnel are required to thoroughly wash their hands upon exiting the greenhouse.
- All procedures shall be performed carefully to minimize the creation of aerosols and excessive splashing of potting material/soil during watering, transplanting, and all experimental manipulations.
- The need to maintain negative pressure should be considered when constructing or renovating the greenhouse.
- The greenhouse floor shall be composed of concrete or other impervious material with provision for collection and decontamination of liquid run-off.
- Windows shall be closed and sealed. All glazing shall be resistant to breakage (e.g.,, double-pane tempered glass or equivalent).
- The greenhouse shall be a closed self-contained structure with a continuous covering that is separated from areas that are open to unrestricted traffic flow. The minimum requirement for greenhouse entry shall be passage through two sets of self-closing locking doors.
- The greenhouse facility shall be surrounded by a security fence or protected by equivalent security measures.
- Internal walls, ceilings, and floors shall be resistant to penetration by liquids and chemicals to facilitate cleaning and decontamination of the area. All penetrations into these structures and surfaces (e.g.,, plumbing and utilities) shall be sealed.
- Bench tops and other work surfaces should have seamless surfaces that are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
- The greenhouse contains a foot, elbow, or automatically operated sink, which is located near the exit door for hand washing.
- An autoclave shall be available for decontaminating materials within the greenhouse facility. A double-door autoclave is recommended (not required) for the decontamination of materials passing out of the greenhouse facility.
- An individual supply and exhaust air ventilation system shall be provided. The system maintains pressure differentials and directional airflow, as required, to assure inward (or zero) airflow from areas outside of the greenhouse.
- The exhaust air from the greenhouse facility shall be filtered through high efficiency particulate air-HEPA filters and discharged to the outside. The filter chambers shall be designed to allow *in situ* decontamination before filters are removed and to facilitate certification testing after they are replaced. Air filters shall be 80-85% average efficiency by the American Society of Heating, Refrigerating, and Air Conditioning Engineers (ASHRAE) Standard 52-68 test method using atmosphere dust. Air supply fans shall be equipped with a back-flow damper that closes when the air supply fan is off. Alternatively, a HEPA filter may be used on the air supply system instead of the filters and damper. The supply and exhaust airflow shall be interlocked to assure inward (or zero) airflow at all times.
- BSL3-P greenhouse containment requirements may be satisfied using a growth chamber or growth room within a building provided that the location, access,

airflow patterns, and provisions for decontamination of experimental materials and supplies meet the intent of the foregoing clauses.

- Vacuum lines shall be protected with high efficiency particulate air/HEPA or equivalent filters and liquid disinfectant traps.

Determination of Plant Containment Level

Knowledge of the organisms and judgment based on accepted scientific practices should be used in all cases in selecting the appropriate level of containment. For example, if the genetic modification has the objective of increasing pathogenicity or converting a non-pathogenic organism into a pathogen, then a higher level of containment may be appropriate depending on the organism, its mode of dissemination, and its target organisms.

Experiments that fall under Section III-E of the *NIH Guidelines* require Institutional Biosafety Committee notice simultaneous with initiation. Those that fall under Section III-D require Institutional Biosafety Committee approval before initiation.

BSL1-P is recommended for most experiments with recombinant DNA-containing plants and plant-associated microorganisms (excluding those covered below for Section III-D). Example: plant transformation using recombinant Agrobacterium where the genetic modification is not expected to increase adverse characteristics.

BSL2-P Biological Containment is recommended for:

- Plants modified by rDNA that are noxious weeds or can interbreed with noxious weed in the immediate geographic area.
- Plants in which the introduced DNA represents the complete genome of a non-exotic infectious agent.
- Plants associated with recombinant DNA-modified non-exotic microorganisms that have a recognized potential for serious detrimental impact on managed or natural ecosystems.
- Plants associated with recombinant DNA-modified exotic microorganisms that have no recognized potential for serious detrimental impact on managed or natural ecosystems.
- Experiments with recombinant DNA-modified arthropods or small animals associated with plants, or with arthropods or small animals with recombinant DNA-modified microorganisms associated with them if the recombinant DNA-modified microorganisms have no recognized potential for serious detrimental impact on managed or natural ecosystems.

BSL3-P Biological Containment is recommended for:

- Experiments involving most exotic infectious agents with recognized potential for serious detrimental impact on managed or natural ecosystems when recombinant DNA techniques are associated with whole plants.

- Experiments involving plants containing cloned genomes of readily transmissible exotic infectious agents with recognized potential for serious detrimental effects on managed or natural ecosystems in which there exists the possibility of reconstituting the complete and functional genome of the infectious agent by genomic complementation in plants.
- Experiments with microbial pathogens of insects or small animals associated with plants if the recombinant-DNA modified organism has a recognized potential for serious detrimental impact on managed or natural ecosystems.
- Experiments involving sequences encoding potent vertebrate toxins introduced into plants or plant-associated organisms.

Biological Containment of Plants

Effective dissemination of plants by pollen or seed can be prevented by one or more of the following procedures:

- Cover the reproductive structures to prevent pollen dissemination at flowering and seed dissemination at maturity;
- Remove reproductive structures by employing male sterile strains, or harvest the plant material prior to the reproductive stage;
- Ensure that experimental plants flower at a time of year when cross-fertile plants are not flowering within the normal pollen dispersal range of the experimental plant; or ensure that cross-fertile plants are not growing within the known pollen dispersal range of the experimental plant.

Biological Containment of Microorganisms Associated with Plants

Effective dissemination of microorganisms beyond the confines of the greenhouse can be prevented by one or more of the following procedures:

- Confine all operations to injections of microorganisms or other biological procedures (including genetic manipulation) that limit replication or reproduction of viruses and microorganisms or sequences derived from microorganisms, and confine these injections to internal plant parts or adherent plant surfaces;
- Ensure that organisms, which can serve as hosts or promote the transmission of the virus or microorganism, are not present within the farthest distance that the airborne virus or microorganism may be expected to be effectively disseminated;
- Conduct experiments at a time of year when plants that can serve as hosts are either not growing or are not susceptible to productive infection; Use viruses and other microorganisms or their genomes that have known arthropod or animal vectors, in the absence of such vectors;
- Use microorganisms that have an obligate association with the plant; or
- Use microorganisms that are genetically disabled to minimize survival outside of the research facility and whose natural mode of transmission requires injury of the target organism or assures that inadvertent release is unlikely to initiate productive infection of organisms outside of the experimental facility.

Biological Containment of Macro-organisms Associated with Plants

Effective dissemination of arthropods and other small animals can be prevented by using one or more of the following procedures:

- Use non-flying, flight-impaired, or sterile arthropods;
- Use non-motile or sterile strains of small animals;
- Conduct experiments at a time of year that precludes the survival of escaping organisms;
- Use animals that have an obligate association with a plant that is not present within the dispersal range of the organism; or
- Prevent the escape of organisms present in run-off water by chemical treatment or evaporation of run-off water

Risk Groups

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

Beginning with RG1 agents, which are nonpathogenic for healthy human adults, the scheme ascends in order of increasing hazard to RG4. The risk group listing of the *NIH Guidelines* is an accepted standard, even when recombinant DNA technology is not used. The American Biological Safety Association (ABSA) also provides a comprehensive risk group listing that references agencies globally. Pathogen Safety Data Sheets (PSDSs) available through the Public Health Agency of Canada (https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment.html) are an excellent source of information about pathogens.

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

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

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

RISK GROUP 4 agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk). Examples: Ebola virus, Herpesvirus simiae (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 adjusted to reflect the specific situation in which the pathogen will be used.

Consideration also is extended to microorganisms that cause diseases in animals and/or plants, which are not categorized into risk groups as are human pathogens. The desired containment for animal and plant pathogens is based on the severity of the disease, its ability to disseminate and become established in the local environment, and the availability of prophylactic treatment.

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

Routes of Infection

Pathogens can be transmitted via several different routes in the laboratory. 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, animal bites, or percutaneous self-inoculation (injection or incision). Precautions should be taken to avoid contamination of cell phones if used in laboratories. Increased risk is associated with pathogens that are aerosol transmitted and when high concentrations or large volumes are used.

Inhalation of infectious aerosols is implicated as the cause of many laboratory-acquired infections (LAI). Even pathogens that normally do not cause infections by inhalation route present a danger when aerosolized. Aerosols can spread throughout the laboratory by traveling along air currents which creates the potential for indirect laboratory acquired infections to occur. Activities that have the potential to create aerosols should be performed in a biological safety cabinet (BSC) whenever possible. The BSC protects the worker and the work environment. If the activity cannot be performed in a BSC, additional personal protective equipment (PPE) such as a respirator may be required.

Opportunistic pathogens are organisms not known to cause infection in healthy individuals but are known pathogens of persons who have been compromised in various ways including:

- Open wounds or cuts
- Antibiotic therapy
- Persons with immunocompromised, immunosuppressed, or susceptible immune status due to infection, acquired or congenital condition, or via therapy (e.g., infants or elderly, pregnancy, diabetes, complement deficiencies, AIDS, severe asthma, bone marrow or organ transplantation, chemotherapy, long-term steroid treatment)
- Exposures to high doses or atypical routes

If work with pathogens is performed in the laboratory, you are encouraged to discuss with Occupational Medicine at University Health Services or your personal physician the agents present in the laboratory.

Clinical and Pathological Specimens

Every specimen from humans or animals may contain infectious agents. Personnel in laboratories and clinical areas handling human blood or body fluids should 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 OSHA to complete bloodborne pathogen training. Bloodborne pathogen training for researchers is available through the Office of Biological Safety. Hepatitis B vaccination is available through Occupational Medicine at University Health Services.

An exposure control plan, which is part of the biosafety protocol, must be prepared by laboratories that handle human blood or other potentially infectious materials (OPIM). OPIM 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, blood, primary cells or established cell lines, HIV- or HBV-containing culture media or other solutions, and cells or tissues from experimental animals administered human-derived material, HIV, or HBV are also included are also subject to oversight. Contact the Office of Biological Safety for more information on precautions and regulatory requirements.

Cultures

When a cell or tissue explant culture is inoculated with or known to contain a pathogen, it should be classified and handled at the same biosafety level as the agent. BSL2 containment and practices are used for human-derived cells (primary cells and established cell lines), all human clinical material (e.g., tissues and fluids obtained from surgery or autopsy), nonhuman primate cells and tissues, and any non-human cells

exposed to or transformed by an oncogenic virus. A biosafety cabinet should be used for manipulations that have potential to create aerosols.

Biological Toxins

Many labs at UW-Madison utilize biological toxins in their research programs, often in conjunction with animals and microbes. Biological toxins are toxic substances that are produced by microorganisms, animals, and plants and are capable of causing harm to other living organisms. Chemicals or products not produced by living organisms are not considered biological toxins but can be equally harmful. Biological toxins (e.g., bacterial toxins, mycotoxins, seafood toxins) handled, stored, or intentionally produced in your laboratory must be listed on laboratory biosafety protocols, depending on factors such as toxicity (LD50). Toxins must be appropriately stored in a designated location and clearly labeled. Toxic synthesized chemicals and antibiotics are not considered to be biological toxins and should not be listed in your biosafety protocol. Contact the Office of Biological Safety if you are unsure whether a material is considered to be a biological toxin or if a material must be listed on your biosafety protocol. OBS will work with laboratories to determine the required biosafety level, handling practices, and inactivation methods for studies involving biological toxins.

Potential exposure routes, toxicity, and effects of exposure vary widely between biological toxins, and may include acute as well as chronic effects. Numerous biological toxins are considered to be potent carcinogens, especially mycotoxins. Symptoms from exposure to some toxins can have a very rapid onset, while symptoms from exposure to other toxins may not be noticeable until years after the exposure. Some toxins accumulate over time and even exposure to very small amounts can be harmful over time, whereas others are rapidly eliminated. Inactivation procedures also vary for specific toxins. It is thus important to understand the specific properties, intoxication routes, and inactivation procedures of a biological toxin prior to use in laboratories. Extreme caution is needed when handling concentrated lyophilized biological toxins as it can create an inhalation risk, even if the toxin is not known to normally cause intoxication via the inhalation route. BSL2 is generally required for handling purified biological toxins, and a BSC, chemical fume hood, or other engineering control is often required, depending on the toxin's specific properties and procedure for its use. Rodents treated parenterally (IV, IM, IP, or subcutaneously) with some biological toxins, in the absence of known pathogens, can often be subsequently housed at ABSL1, depending on the toxin's properties.

Some high-risk biological toxins are considered Select Agents. To learn which biological agents and toxins are considered Select Agents, visit the following website: **http://www.selectagents.gov/SelectAgentsandToxinsList.html**. Please contact OBS if you are unsure whether a biological toxin in your lab is considered a Select Agent toxin. Select Agent biological toxins are exempt from the Select Agent regulations if the total quantity under the control of a PI falls below a certain threshold: **https://www.selectagents.gov/sat/permissible.htm**
OBS will provide information about required inventory and storage regulations to laboratories possessing sub-threshold amounts of Select Agent toxins.

Animals

Exercise care and thoughtfulness when using animals in research. Numerous risks may be present when animals are used in studies of microorganisms and an investigator will need containment and PPE that protects against the biological hazards. Precautions commonly include use of a lab coat, gloves and eye protection when handling animals and their bedding; respiratory protection may be recommended when specific conditions present a concern.

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

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

Transfer of human cells, primate cells or opportunistic microbes, whether newly isolated or well-established, into immunocompromised animals could result in propagation of pathogens that would be suppressed in the normal host. ABSL2 containment must be applied to mitigate against such risks and also to prevent spread of animal pathogens within a research colony.
Mixed waste disposal methods require a thorough risk assessment. Please contact the Office of Biological Safety for assistance.

Aerosol Generating Activities

Routine manipulations of biological materials may also release hazardous agents via aerosol formation. Examples include:

- Removing stoppers from culture vessels
- Opening vessels after vigorous shaking or vortexing
- Flame-sterilizing utensils
- Electroporation
- Centrifugation
- Sonicating, homogenizing, blending, or grinding tissues
- Pipetting
- Animal inoculations
- Surgery or necropsy
- Tissue sectioning

- Flow cytometry

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 biosafety cabinet. Accidental spilling of liquid infectious cultures is an obvious hazard due to the generation of aerosols (airborne droplets containing microorganisms). See biohazard spill information for response and containment of accidental spilling on Office of Biological Safety website. (https://ehs.wisc.edu/labs-research/biological-safety/biological-spill-protocols/)

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

Personal Protective Equipment

In research laboratories at UW-Madison, it is necessary to wear closed-toe shoes and clothing that covers the leg down to the shoe. Individual labs may have additional clothing requirements depending on activities. For example, may require the use of lab-dedicated clothing (scrubs or personal clothing) kept solely to wear in the laboratory that does not “go home” unless decontaminated.

Laboratory coats provide a removable barrier that protects the worker’s skin and clothing from hazardous materials in the laboratory. To avoid bringing hazardous materials out of the laboratory, lab coats are removed before exiting and remain in the laboratory. Lab coats should be laundered onsite or through a laundry service and never taken home for cleaning. Laboratories laundering lab coats, scrubs or other laboratory clothing, and linens (e.g., towels, drapes, blankets), in house using a washer and dryer should not sort the materials once placed in the designated laundry container prior to laundering. The washer and dryer should be dedicated for use only with laundering lab coats, scrubs or other laboratory clothing, and linens (e.g., towels, drapes, blankets). Signage is placed on the machines indicating that they are dedicated, and the room has restricted access. In house laundering is described in the biosafety protocol and depending on the biological materials used in the lab, laundering parameters may need to be specified (e.g., water temperature, detergent or disinfectant used) and decontamination may be required prior to laundering (e.g., autoclave, chemical disinfection).

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 in a manner that prevents contamination of hands.

Gloves should be removed before exiting the laboratory. Disposable gloves should not be reused.

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. University Health Services (UHS) offers prescription safety glasses to help employees comply with the OSHA Lab Standard.

The background level of microbes in the research laboratory should be negligible when good microbiological techniques are employed. 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. A concern regarding respiratory protection is that, if used improperly, the user has a false sense of security. A surgical mask or common dust mask 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 (PAPR) is used when respiratory protection is critical for work with highly pathogenic microbes or in situations where a biosafety 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. Contact the Environmental and Occupational Health Office for guidance on appropriate respiratory protection.

Depending on the nature of the work, protective clothing could also include disposable sleeves, coats that close in back, disposable protective suits (e.g., Tyvek), hair bonnets, and shoe covers.

LABORATORIES

Engineering Controls

Table 2 describes the relationship between biosafety levels and engineering controls, which include laboratory design, laboratory 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 and should be contacted when changes (i.e., addition, deletion, or relocation) of protective

equipment occurs and when planning renovations projects that affect laboratory ventilation.

Offices or other non-laboratory spaces should not be located within a laboratory. Please contact the Office of Biological Safety for information on laboratory design.

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 laboratories should not be recirculated into other parts of the building. It should be ducted to the outside and released from a stack remote from the building air intake. In certain special situations, air exhausting from a hazardous facility should be filtered through certified HEPA (high efficiency particulate air) filters that are tested at least annually and verified to retain microorganisms.

Table 2. Summary of Standards For Facilities and Equipment For Biosafety Levels

Key: -- not applicable or needed	BSL 1	BSL 2	BSL 3
Laboratory visit by Office of Biological Safety	Desirable	Yes	Yes
Isolation of laboratory from public areas	---	---	Desirable
Eyewash, plumbed	Yes	Yes	Yes
Interior surfaces (impervious, cleanable):	Yes	Yes	Yes
Bench tops	Yes	Yes	Yes
Laboratory furniture	Yes	Yes	Yes
Floors, non-absorbent	Yes	Yes	Yes
Floors, seamless with integral cove base	---	Desirable	Yes
Ceiling, conventional or suspended	Yes	Yes	---
Ceiling, permanent and sealed	---	---	Yes
Sinks in laboratory	Yes	Yes	Yes
Hands-free	---	---	Yes
Water supply protected	---	---	Yes
Windows allowed	Yes	Yes	Yes
May be opened	No	No	No
Must be sealed	No	No	Yes
Room penetrations sealed for gas decontamination (pressure decay testing)	No	No	Desirable
Ventilation (supply/exhaust not recirculated)	Yes	Yes	Yes
Inward air flow (negative pressure)	Yes	Yes	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):	Yes	Yes	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[a]
Waste effluent treatment	---	---	Desirable
Centrifuge with sealed rotors, buckets or safety cups, HEPA filter required	---	Desirable	Yes

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

Biocontainment Equipment

Information regarding biocontainment equipment (BSC, ATS, and CAD) can be found in The UW-Madison Manual for Biosafety Cabinets, Animal Transfer Stations, and Clean Air Devices. https://ehs.wisc.edu/wp-content/uploads/sites/1408/2020/11/EHS-BIO-GUI-013_V01.pdf Information in this manual includes the types of containment equipment, how they work, what protection they provide, their use with biological, chemical, and radiological materials, and services provided by the UW-Madison BSC Certification Program. Requirements for the purchasing, acquisition, certification, relocation, and disposal of biocontainment equipment are found within the IBC BSC Policy. https://policy.wisc.edu/library/UW-6088

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. Decontamination, inactivation, and disposal procedures outlined in laboratory biosafety protocols must be followed. The appropriate packaging of all waste is fundamental for assuring protection of the handler and proper disposal. A display poster that summarizes sharps and glass disposal is available at the EH&S website and upon request.

Decontamination is defined as the process of reducing the number of disease-producing microorganisms and rendering an object safe for handling.
Disinfection is defined as a process that kills or destroys most disease-producing microorganisms, except spores.
Sterilization is defined as a process by which all forms of microbial life, including spores, viruses, and fungi, are destroyed.

Types of Biohazardous Waste

The following items require decontamination or inactivation prior to disposal:

- Microbiological laboratory waste: Cultures derived from clinical specimens and/or pathogenic microorganisms.
- Medical waste: Tissues, liquid blood, cells and body fluids from humans. Exceptions are urine, saliva, tears, sweat, or feces from humans that are not anticipated to contain pathogens and not visibly contaminated with blood.
- Zoonotic waste: Tissues, liquid blood, cells, body fluids, and bedding from an animal that is carrying an infectious agent that can be transmitted to humans.
- Recombinant waste: Recombinant organisms, recombinant DNA/RNA.

- Exotic or virulent plant and animal pathogens.
- Sharps waste: Contaminated medical and nonmedical sharps
- Biological toxins
- Plant waste: All portions of exotic and non-endemic plants

Mixed Waste

Please contact Environment, Health and Safety with disposal questions for biological materials mixed with hazardous chemicals and/or radioisotopes.

Medical Sharps

Medical sharps are instruments designed to cut or penetrate skin. Examples include syringes with needles, stand-alone needles, lancets, and razor blades, regardless of their actual use. These items must be disposed of in a puncture-resistant, ASTM-certified medical sharps container. Do not overfill beyond the "fill" line on the container. Note that even if these items are unused and in their original packages/sleeves, they still must be properly disposed of in a medical sharps container.

When filled and ready for disposal, securely close the lid. Medical sharps require special handling and may not go directly to the landfill. Thus, do NOT put an "OK to Trash" sticker on medical sharps containers. A contractor, MERI (Madison Environmental Resourcing, Inc.), processes medical sharps by grinding followed by microwave inactivation. All medical sharps containers at UW-Madison must be disposed of through MERI. Medical sharps need not be autoclaved prior to disposal by MERI unless generated or used by a BSL3 or Select Agent facility. If you plan to autoclave the sharps container, make sure it is made from heat resistant material. Please note that general building custodial personnel are instructed not to handle or transport sharps containers as part of their safety training. Typically, labs must take the containers to the nearest MERI collection bin, often in loading docks.

Non-Medical Sharps

Non-medical sharps are lab materials that can cut, or puncture the skin, but are not intended to do so. Examples include fragile glass, glass slides and cover slips, metal wires, pipets, and pipette tips. These items must be disposed of in a manner that prevents harm to others. They must be decontaminated prior to disposal if used with infectious agents or recombinant materials.

Infectious, Microbial and Recombinant Waste

Materials from laboratories and animal facilities, such as cultures, tissues, media, and plastics contaminated with biohazardous, potentially biohazardous, or recombinant substances must be decontaminated before disposal. Collect contaminated materials in leak-proof containers labeled with the universal biohazard symbol; autoclavable biohazard bags are recommended. After decontamination, a green "OK to Trash" sticker should be affixed to the bag to show that the material is decontaminated and safe to handle by UW custodial/waste disposal personnel. If bio-waste being placed in autoclave bags includes materials such as pipet tips or plastic serological pipets that

may poke poles in the bag, the autoclave bag should be placed into a sturdy secondary box, sealed and affixed with an “OK to Trash” sticker.

Red biohazard bags

Effective 07/2011, UW’s waste contractor will NOT accept any red biohazard waste bag or containers because they consider these waste items to be Medical Waste. Red biohazard bags can only be used with biological waste that will be disposed of through MERI. Note that orange or clear biohazard waste bags can also be used for biological waste going to MERI. This waste typically does not have to be autoclaved prior to placing in MERI containers. Do NOT place an “OK to Trash” sticker on items being disposed of through MERI. In most buildings, laboratory staff must transport red biohazard bags to the nearest MERI collection bin.

Clear or orange biohazard bags

Clear or orange biohazard bags (not red) can be used for biological waste that will be autoclaved and then sent to the landfill. Once orange or clear biohazard bags are autoclaved, place a green “OK to Trash” sticker labelled with your name and room number on each bag and place them at a designated pickup location in your facility for subsequent pickup by custodial staff.

Facility-specific exceptions

Be aware that there are buildings on campus (such as UW Hospital and Clinics) that may have different waste pick-up policies than those stated here for custodial personnel. Be aware of your building policies and contact the facilities manager in your building. If your facility is off the main UW-Madison campus, be sure to verify disposition procedures for your sharps and infectious waste as they may differ from buildings on the main campus; building managers may be your best resource.

Liquid Waste

Liquid waste that is contaminated with infectious agents or biological toxins must be rendered safe by chemical or autoclave treatment before sewer disposal. Care must be taken to avoid splashing and generating aerosols. Sewer lines should be decontaminated by flushing with hypochlorite (1:10 dilution of household bleach containing 5.25%-6.15% sodium hypochlorite) prior to servicing.

Waste from Animal Experiments

Animal waste (e.g., bedding, feces, urine) may require disinfection/inactivation by methods as described in your biosafety protocol followed by disposal via trash or sanitary sewer. Animal carcasses are to be disposed by incineration or via chemical digestion. Disposal outside of these regular routes must be detailed in biosafety and/or animal protocols.

Animal waste which does not require disinfection/inactivation as described in the biosafety protocol, is disposed via trash or sanitary sewer. Disposal outside of these regular routes must be reviewed and approved.

All animal carcasses covered under Appendix M of the *NIH Guidelines* (containing recombinant or synthetic nucleic acid molecules or a recombinant or synthetic nucleic acid molecule-derived organism) should be disposed of by incineration or chemical digestion to avoid its use as food for human beings or animals unless specifically authorized by an approved Federal agency.

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. Non-infectious waste may include:

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

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 Environment, Health and Safety 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, non-hazardous laboratory waste should be disposed of

routinely as much as possible so as to reduce the amount of waste requiring special handling.

Considerations for Shelf Life for Disinfectants

The stability of chemical disinfectants is impacted by several factors, such as dilution, pH, temperature, and humidity of storage conditions. Because the efficacy of disinfectants is important, we recommend that you consider the following for chemical disinfectant shelf life:

- Natural breakdown of chemical disinfectants may occur impacting effectiveness. Not all disinfectants have the same shelf life.
- Ideal condition storage conditions are specified by the manufacturer and deviations in conditions may impact efficacy.
- Manufacturers may place codes on the chemical disinfectant indicating the date produced. Check the manufacturer website for more information.
- Once a chemical disinfectant container is opened, the manufacturer may recommend an expiration date. This is important when considering stock disinfectants. Check the manufacturer website and label the container with the expiration date.
- Once diluted and in use in the lab, label with date made and an expiration date. This label is in addition to the hazard communication label.

Steam Sterilization

Decontamination is best accomplished by steam sterilization in a properly functioning autoclave that is efficacy tested monthly with biological (i.e., *Bacillus stearothermophilus* spore testing) or chemical (i.e., 3M™ Comply™ SteriGage™) indicators that verify adequate temperatures and times have been reached inside the material/load to kill microorganisms. Efficacy test results must be recorded and retained, preferably on or near the autoclave. Indicator tape provides assurance only that a high temperature was reached; it does not indicate if 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 autoclaving all biohazardous wastes for at least one hour is recommended, 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, manure, or soil, one hour may be insufficient to inactivate certain pathogens buried within. A considerably longer exposure time (e.g., 8 to 12 hours) may be required to effectively decontaminate such waste. General autoclave safety and use guidelines are available through the OBS website.

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 is generally greater for enveloped viruses than for non-lipid viruses, and greater for vegetative bacteria and fungi than for spores. Mycobacteria are more resistant to inactivation than most bacteria, while prions are notably resistant to most chemicals.

The following brief overview cannot do justice to the complexity of this subject. Additional references should be consulted, and testing done to verify the efficacy for a 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 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 handwashing 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 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 Environment, Health and Safety 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%-6.15%. NaOCl Solutions should be stored in an opaque bottle to reduce decay during storage. A freshly prepared solution should be used for sanitary purposes such as cleaning a blood spill. Solutions containing bleach should not be autoclaved as chlorine gas will be released. Also be aware that using chlorine compounds to disinfect substances co-contaminated with radioiodine may cause gaseous release of the isotope. Contact with skin should be avoided. Example products: Clidox, Clorox, or other household bleach.

Iodophors, complexes of iodine and carrier, have good germicidal properties with relatively low toxicity and irritancy. Efficacy has been demonstrated against bacteria

including mycobacteria, viruses, and fungi; prolonged contact time may be needed to kill certain fungi and bacterial spores. Example products: Povidine, Betadine.

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

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

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

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

Incineration

The optimal method of disposal for some types of waste is incineration. Animal carcasses are routinely picked up by the EH&S Department for disposal by this method. Bedding, plastic, and metallic objects must be excluded from packages of animal carcasses. Consult the EH&S Department for more information.

UV Treatment

UV light is only effective on surfaces it contacts, has little ability to penetrate materials, and the UV output decreases as the lamp ages. Each week UV lamps must be cleaned to remove dust and fingerprints that may block the germicidal effectiveness of the ultraviolet light, and the UV intensity must be checked with a UV meter. Personnel should avoid exposure to light in this wavelength region since brief exposure can cause erythema (sunburn) and eye injury. The efficacy of UV light for disinfection is limited by a number of factors (e.g., age, cleanliness, temperature, humidity) and thus UV lights cannot be used as the only disinfection method. Additionally, the use of UV lights in BSCs is not recommended by the CDC and the NIH.

EMERGENCY PLANS

Emergency plans should be tailored for the laboratory. The laboratory supervisor should prepare instructions specifying immediate steps to be taken and all personnel should understand basic emergency measures. It is recommended the instructions are displayed prominently in the laboratory and annually reviewed with personnel. No single plan will apply to all situations, but the following general principles should be considered:

- Always know the location of emergency response materials, such as spill kits, fire extinguishers, eyewashes, safety showers, first aid kits, automated exterior defibrillators (AED), contact numbers and first aid kits.
- Attention to immediate personal danger overrides containment considerations.
- If necessary, call 9-1-1 or UW Police.
- Fire, security, and police personnel may enter a BSL1 or BSL2 laboratory as they are adequately prepared to enter BSL1 and BSL2 biological laboratories. For BSL3 biological laboratories, a lab-specific plan needs to be in place for emergency personnel.
- The supervisor should always be notified.
- A First Report of Biological Exposure or Release Form is prepared within 24 hours even in situations that do not involve emergency responders or require immediate medical care.
- Notify the Biological Safety Officer of any spills outside containment, potential exposures, violations of the *NIH Guidelines*, or any research-related accidents and illnesses.

Exposure Response

PIs are asked in the context of the biosafety protocol to consider the consequences of an accidental exposure to agents (e.g., microbes, DNA, toxins) used in their research and prepare an appropriate response procedure. Occupational Medicine provides medical response plans for some agents on their website https://www.uhs.wisc.edu/eoh/occupational-medicine/. A sample general procedure can be found at: https://ehs.wiscweb.wisc.edu/wp-content/uploads/sites/25/2017/01/BiologicalExposureAndSpillResponseGeneral.pdf. 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. For any possible or identifiable exposure (e.g., ingestion, skin puncture, inhalation) to a hazardous substance, individuals must seek immediate medical assessment:

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

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 agent(s) and the signs and symptoms of infection or intoxication
- Distinct characteristics of the laboratory strain(s), such as known antibiotic resistance, transmissibility, atypical tissue tropism, foreign genes that alter pathogenicity, and so forth.
- Recommendations for treatment regarding effective drugs, quarantine, and so forth.
- A test to establish a history of exposure at the start of employment and periodically thereafter may be appropriate for work with a few pathogens such as *Mycobacterium tuberculosis*.
- Completion of a First Report of Biological Exposure or Release Form, located online at the OBS website (https://ehs.wisc.edu/first-report-of-biological-exposure-or-release-event/) within 24 hours.

Biohazardous Spills

Laboratories should be prepared to immediately address biohazardous spills by training personnel in advance and having appropriate spill-control materials in place. Note that biohazardous materials being transported outside of laboratories, including to autoclaves, should be in secondary containment capable of completely containing the spills.

In addition to spill-prevention procedures, information regarding spill-control procedures should be displayed in laboratories and periodically reviewed with personnel. In the event of emergency, **do not hesitate to call 911 if necessary**. The Office of Biological Safety is available for additional assistance and information at 608-263-2037. More emergency contact information is available at https://ehs.wisc.edu/emergencies/

All spills or releases of biohazardous or recombinant materials must be reported to the Principal Investigator (PI) and also to the Office of Biological Safety (OBS) and University Health Services (UHS) within 24 hours through the use of the First Report of Biological Exposure or Release Event Form, available on the EH&S website: https://ehs.wisc.edu/first-report-of-biological-exposure-or-release-event/

Recommended Supplies

Appropriate materials to handle biohazardous spills should be prepared in advance, placed in strategic locations inside or outside the laboratory, and all laboratory personnel informed of the location(s). The items that are generally recommended include, personal protective equipment (PPE), absorbent materials, disinfectant(s), clean-up tools, and signage. More details on preparing a spill kit can be found on the Office of Biological Safety website: https://ehs.wisc.edu/wp-content/uploads/sites/1408/2020/10/EHS-BIO-GUI-028-V01.pdf

Decontamination/Spill Procedures

Laboratory general or specific spill cleanup procedures for spills inside containment (e.g., in a BSC) and spills outside containment that are suitable for posting in the laboratory can be generated using a template from the Office of Biosafety https://ehs.wisc.edu/wp-content/uploads/sites/1408/2021/03/EHS-BIO-GUI-011-V02.pdf. There is also a standard spill protocol found at: https://ehs.wiscweb.wisc.edu/wp-content/uploads/sites/25/2017/01/BioHazSpillProtocolSample.pdf

Spills in BSL3 Laboratories

Laboratories handling materials at BSL3/ABSL3 can use the above protocols as a starting point but should work closely with OBS to develop specific spill protocols for their agents for each area. These protocols must be included in their laboratory biosafety protocol and BSL3 manual.

TRANSPORT OF HAZARDOUS MATERIALS

Transport of hazardous materials on campus, in a campus vehicle or by a commercial carrier (such as FedEx or UPS) requires special attention to particular safety containment and regulations.

To protect the public at large, hazardous materials transportation by commercial carrier is regulated by the US Department of Transportation (DOT) as well as by the International Air Cargo Organization (ICAO) and International Air Transport Association (IATA). The US Department of Transportation (DOT) regulates the shipping and transportation of hazardous materials in commerce on United States' roadways, airways and vessels as described in the Code of Federal Regulations Title 49, Parts 171 to 178 (49CFR §171-178). Air transport and international transport of Dangerous Goods (aka Hazardous Materials) in commerce is regulated by International Air Cargo Organization (ICAO) and International Air Transport Association (IATA). International and DOT regulations are similar but can vary on some substances; therefore, it is crucial to become trained and certified according to both regulatory bodies.

A hazardous material is defined as "a substance or material that the Secretary of Transportation has determined is capable of posing an unreasonable risk to health, safety, and property when transported in commerce." All DOT hazardous materials regulated in transport are listed in the 49CFR §172.101 Hazardous Materials Table.

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

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

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

OBS provides Bio-Hazardous Materials Shipping certification training through a combination of online and in-person courses. Training through OBS is good for 2 years. See the EH&S website Hazmat Shipping page (https://ehs.wisc.edu/labs-research/hazmat-shipping-and-transportation/) or contact OBS for additional information.

Although UW is exempt from US DOT Hazardous Materials transportation regulations (CFR49) when moving materials on campus or in a campus vehicle on roadways, we must still adhere to safety guidelines which essentially mimic the HazMat regulations. OBS and EH&S published guidelines for transport of biologicals and other hazardous materials on campus or by campus vehicle: https://ehs.wisc.edu/labs-research/hazmat-shipping-and-transportation/

OBS and EH&S strongly advise against individuals transporting hazardous materials in a personal vehicle, as your personal insurance may not cover incidences occurring while moving these materials (materials include but are not limited to pathogenic specimens, dry ice, liquid nitrogen, chemical preservatives, or other laboratory chemicals). It is also recommended that hazardous materials are not transported via taxi, city bus, bicycle, or scooter either on or off campus by personnel.

Transport of Hazardous Material for Disposal

Movement of hazardous materials for disposal (i.e., waste) on campus must only be performed by UW EH&S employees or approved contractors. Hazardous waste is regulated by the US Environmental Protection Agency (EPA) and requires specialized training to perform appropriate handling, marking and documentation. UW EH&S has designated employees trained for handling, transporting and disposal of hazardous materials. For additional information or to request disposal services: https://ehs.wisc.edu/disposal-services/

Import/Transport and Related Permits

The acquisition of some research materials from outside the U.S. and in some cases from outside Wisconsin may raise concern with federal agencies tasked with protecting agriculture (USDA) or human health (CDC). Thus, sometimes an import/transport permit is needed prior to obtaining these materials. Materials of concern are typically from animal, plant, or human sources, and the permitting requirements and exceptions change frequently. The Convention on International Trade in Endangered Species (CITES) regulates international trade in materials from endangered species, including tissues and cell lines from many primate species. OBS can provide guidance about whether a permit or specific language on shipping documentation is needed prior to obtaining or sending certain materials. Note that Material Transfer Agreements (MTAs),

while often related to incoming or outgoing shipments of research materials, are not negotiated by EH&S. Information on MTAs can be found at https://www.rsp.wisc.edu/contracts/mta.cfm and https://kb.wisc.edu/hsirbs/page.php?id=50265

LABORATORY SECURITY AND PUBLIC AREAS

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:

- Wear visible identification
- Remove sensitive data from the Web
- Report suspicious activities and unauthorized individuals
- Unknown person SOP
- Do not prop doors open; lock doors when no one is present, keep keys in a secure place out of sight

The degree to which laboratory security is implemented should be commensurate with risk. All laboratories, including those handling only low-risk biological materials under BSL1 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 laboratories and to ask their purpose. According to CDC's guidance for BSL1 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 all laboratory materials 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. Equipment should be located in the laboratory to prevent theft and release of materials. For materials stored outside of the laboratory, such as in a freezer located in a hallway or shared equipment space, the equipment must be locked at all times.

SPECIAL CONSIDERATIONS

Considerations for Working Alone in Laboratories

Specialized work environments like laboratories can pose risks which may be increased when working alone. Individuals working with hazardous materials or performing hazardous tasks ordinarily should not work alone. If a lab member must work alone (for example, after hours or in a laboratory space with restricted access), the laboratory should perform a risk assessment of the work area, materials, and activities to identify any potential or existing hazards and implement risk mitigation.

Laboratories should consider the following:

- Implement a call in/out process
- Define specific tasks not performed while working alone
- Develop emergency plan for persons working alone
- Review expectations, procedures, and processes with all staff

More information may be found in the UW System Administrative Policy 620 Working in Isolation.

Considerations for Training Animal Caretakers

When laboratories partner with a campus vivarium to care for their animals, hazard communication is necessary. This happens at all biosafety levels (BSL1, BSL2 and BSL3). Training for animal caretakers includes information regarding what was administered to the animal, recombinant modifications (animal or biological material), microbe route of transmission if applicable, required PPE, appropriate containment for animal husbandry or veterinary activities, disposal/disinfection methods for bedding and cages, PPE, occupational health requirements, release reporting requirements, and efficacious disinfectants. Laboratories are responsible for training persons taking care of their research animals. This may be accomplished through in person training or other method as described in the approved biosafety protocol.

Considerations for Training Greenhouse Staff

Laboratories using greenhouse space on campus should communicate with the greenhouse manager and plant caretakers regarding the specifics of the research materials. This should occur at all plant biosafety levels (BSL1-P, BSL2-P, and BSL3-P). Training for greenhouse staff and plant caretakers includes information on the plants including any genetic modification(s), insects/animals/microbes used in associated with a plant and any genetic modification(s) they have, biological materials administered to the plants and their route of transmission or dissemination, exotic/non-exotic nature of the materials, plant or pest containment strategies, PPE, disposal/disinfection methods, and release reporting requirements. Training and hazard communication may take place in person or other method as described in the approved biosafety protocol.

Considerations for PPE Disposal

Disposable laboratory, vivarium, or greenhouse PPE that is contaminated or potentially contaminated with biological hazards is disposed in a manner that protects others from exposure and prevents biological material release. Laboratory, vivarium, and

greenhouse environments where disposable PPE is used with biologicals should consider their PPE to be contaminated or potentially contaminated and decontaminate it prior to disposal. Please check your approved biosafety protocol for disposal methods.

Considerations for Teaching Laboratories

A teaching laboratory/space is unique and requires consideration from a number of angles. This is a specialized environment where students are exposed to scientific techniques, experimental design and basic biosafety is integrated into the curriculum. Adding biosafety to the teaching curriculum not only promotes a safe environment, but also enhances the comprehensive experience and education of the students.

Space is an important factor in the teaching laboratory and an assessment of the space should be performed by OBS and/or engineering professionals. During the visit, the following will be assessed:

- Surfaces and flooring
- HVAC
- Containment equipment
- Shared space issues
- Sink position for handwashing
- Eye wash availability and testing
- Shower position for emergencies
- Storage availability for student personal effects

In preparation for the course, PIs/instructors need to consider the following when designing the curriculum and choosing the experiment:

- Hazard communication plan and emergency plan
- Additional biosafety resources for students, instructors and supporting staff
    - https://ehs.wisc.edu/biosafety-training/
    - Additional resources listed in the end of this document
- Training requirements for students, instructors and/or assistants:
    - Emergency and spill procedures
    - Reporting requirements (Lab acquired infections, spills, releases, potential exposures)
    - PPE requirements, availability, and use
    - Disposal and decontamination
    - Sharps (if applicable)
    - Containment equipment training/use (decontamination, function, failure, emergency response)

Some general biosafety guidelines for the teaching laboratory include:

- Wear closed toed shoes
- Wear clothing covering legs
- Wear eye protection
- Wear lab coat
- Signage

- Handwashing after experiment and before leaving the laboratory
- Decontamination of surfaces, equipment, and microbes
- Keep exits and emergency equipment clear
- Post emergency response procedures (potential exposures, spills)
- Personal items should be stored outside of the laboratory
- No food or drink in laboratory

If experiments performed in the teaching labs are subject to the NIH guidelines, a biosafety protocol may be required. In the absence of recombinant work, a biosafety protocol is recommended for laboratory courses and can be reviewed by the IBC to ensure proper biosafety precautions are taken. The Office of Biosafety is here to help with this as well as help provide basic training to the class.

Several helpful resources include:
The CDC Biological Risk Assessment Worksheet: https://www.cdc.gov/csels/dls/bio-risk-assessment.html
The CDC poster:
https://www.cdc.gov/salmonella/pdf/cdc_lai_prevention_poster_012313_508.pdf

Considerations for Core Facilities

A core facility provides training, services, and/or access to specialized equipment. A core facility is generally under the oversight of a PI or department and has personnel dedicated to performing core procedures, coordinating use of the facility, training users, and overseeing the facility.

As with any research laboratory space, there are special considerations for core facilities. A core facility's biosafety protocol should describe (but is not limited to) waste handling, hazard communication (to core personnel, persons operating equipment, and persons entering the facility), disinfection/decontamination of surfaces and equipment, how to handle emergencies and spills, PPE requirements, training for personnel or persons operating equipment, and specific materials that are not allowed in the facility.

Core facilities can expect the space to be listed on biosafety protocols for persons trained to use the equipment, and these protocols are appropriately reviewed and approved by OBS/IBC.

The intake process for the core facility (for cores that are service oriented/accept samples/materials **AND** for cores that are overseeing equipment for people to use) must be described in the core facility's biosafety protocol. This process must ensure that appropriate information is given to core personnel and should be documented by the core. Essential elements of the intake process include:

- Material description (source, modifications, antibiotic resistance if applicable, live/fixed, biosafety level, etc.)
- Contact information for PI and/or lab personnel
- Appropriate method of disinfection/inactivation of materials after experiment is complete

- Appropriate methods for disinfection/inactivation of materials prior to arrival at core facility (e.g., method of fixation, validation of fixation method, etc.)
- Appropriate methods/SOPs for disinfection/inactivation for spills or emergency events
- Occupational health considerations as applicable (e.g., respirator use fit testing, vaccinations)
- Clearly defined expectations for entry and/or use of equipment (e.g., training requirements, PPE, disposal of waste, disinfection/inactivation, emergency response, contact information for core staff in case of questions or emergencies, etc.)
- Other special information as needed
- Informs PI and/or lab personnel of space or equipment instructions or special information
- Informs PI and/or lab personnel that core personnel may request the biosafety protocol and can connect with OBS with any questions or concerns
- Informs PI and/or lab personnel that core staff may refuse materials or services

In addition to the items for the UW-Madison intake process listed above, core facilities accepting samples from non-UW entities or granting access to persons from non-UW entities should also consider the following:

- Intake process should inform persons from non-UW entities of transport expectations for UW campus and UW buildings
- Intake process should inform/define training requirements for persons from non-UW entities
- Training provided for core facility staff on special requirements for non-UW entity persons/samples:
  - Biosecurity considerations (i.e., unattended people or materials, storage of materials, escort for persons)
  - specific core requirements (training, hazard communication expectations, other specific items as outlined in biosafety protocol or intake form)
  - transport and decontamination/disinfection expectations
  - other as specific to Core Laboratory

Considerations for Adding Activities Performed in a Core Facility to the Biosafety Protocol

When research activities are performed in a core facility, the core facility location(s) are listed in the user's biosafety protocol. The research description includes information on which biological materials will be handled in the core facility. In addition, the research team should specify if laboratory personnel will perform activities in the core facility or if materials will be transported to the core facility and core staff will work with the biological materials.

Considerations for Collaborative Research

Collaborative research projects may be described in the biosafety protocol of either collaborating PI. All of the personnel, materials, activities, and locations for a single

experiment or step in an experiment must be on the same biosafety protocol. For example, Investigator A is preparing a microbe stock for Investigator B and Investigator B is administering the microbe to cells.

Investigator A's biosafety protocol would include:

- Personnel that will prepare the microbe
- Microbe and any biological materials needed for preparation of stock (e.g., drug traits, genes, DNA/RNA fragments, or constructs introduced)
- Activities involved in all steps of preparation of the microbe including aerosol generating activities, disinfection/inactivation, transport, spill and emergency procedures, and description of experiments
- All locations where activities involved in preparation of the microbe occur (e.g., laboratory rooms, autoclave location)

Investigator B's biosafety protocol would include:

- Personnel that handle or administer the microbe and cells
- Microbe, cells, and any other biological materials needed for experiment
- Activities involved in culture and infection of cells including aerosol generating activities, disinfection/inactivation, transport, spill and emergency procedures, description of experiments
- All locations where activities involved in culture and infection of cells occur (e.g., laboratory rooms, autoclave location)

Considerations for Large Scale Activities

The *NIH Guidelines* defines Large Scale research as greater than 10 liters of culture in one vessel. Large Scale Research adheres to III-D-6 and Appendix K of the *NIH Guidelines*. If the large scale activities do not involve recombinant materials, OBS will generally follow the same risk assessment process as with recombinant materials and provide the research team with a comprehensive picture of the overall risk and mitigation for the large scale activities. Large scale activities not involving recombinant materials may require IBC review.

To assess the risk of large scale activities, a multifactorial approach is necessary. The agent (its form including modifications, risk group, concentration, etc.), the processes/activities utilized during experimentation as well as the environment/space for the activities, experience and training of personnel are part of the risk assessment.

Space assessment is important when designing large scale activities. HVAC, facility/space constraints and location, containment equipment, and properties (e.g., surfaces, flooring, drains, windows, etc.) for the specific research are evaluated. A visit from OBS prior to starting work is necessary. During the visit, activities, space, details of the spill protocol as well as training of staff is discussed.

A large-scale spill protocol is required large-scale research and elements of the protocol should include:

- Secondary containment (large enough to contain all of the material)
- Decontamination procedures

- Appropriate disinfectant and concentration (an SOP may also be needed for preparation of large amounts of the disinfectant)
- Space specific considerations
- PPE utilized
- Decontamination and disposal of tools/materials utilized or generated in the spill cleanup process

The spill protocol is uploaded to the biosafety protocol and reviewed by the IBC.

At the end of the experiment, decontamination of materials and space will need to occur. This should be described in your biosafety protocol.

SERVICE GUIDANCE

BSL1 and BSL2 Laboratories

Research laboratories and teaching laboratories should prepare for service work or equipment repair (e.g., HVAC, plumbing, electrical, equipment) with careful consideration to a number of factors. The following are recommended for BSL1 and BSL2 laboratories that need service work:

At Any Time (Before/During/After Service Work):

Office of Biological Safety

- Provides resources and fields questions and/or consults with core and laboratory personnel.

Physical Plant/Service Provider

- Service performed in a laboratory space should be scheduled in a timely manner with the Principal Investigator and/or laboratory manager and building manager.
- Prior to entering an animal facility, check in with facility manager to ensure room order and/or rules for entering a facility are followed (i.e., no other rodent exposure the same day)
- Service personnel may be asked to reschedule the work if service impacts laboratory activities

PI or Laboratory Manager

- Ensure that all laboratory personnel are aware of the service and schedule.
- Ensure that all biohazardous waste is removed from the immediate and surrounding space near the service area
- Samples and activities/general lab work should be reduced or stopped until maintenance work is complete (e.g., immediate and surrounding space near the service area).
- Make service area accessible by removing clutter, moving equipment, glassware, chemicals and other lab supplies away from the immediate service area.

- For the immediate and surrounding space designated for service, decontamination performed as follows:
    - Thorough surface decontamination with an approved disinfectant as outlined in biosafety protocol
    - Laboratory personnel are encouraged to communicate the decontamination procedure and specific area/material decontaminated to the service personnel
    - Laboratory personnel are encouraged to document the decontamination
    - Laboratory personnel are encouraged to utilize the “OK to Repair” and “OK to Move” stickers available from OBS

High Containment (BSL3) Laboratories

BSL3 laboratories should prepare for service work or equipment repair with careful consideration to a number of factors. We recommend the following for high containments laboratories:

At Any Time (Before/During/After Service Work):

Office of Biological Safety

- Provides resources and fields questions and/or consults with core and laboratory personnel.

Before Service Work:

Physical Plant/Service Provider

- Service performed in a laboratory space should be scheduled in a timely manner with the Principal Investigator and/or laboratory manager and building manager
- Service personnel maybe asked to reschedule the work if not previously arranged with laboratory and building manager
- Service personnel may be asked to adhere to specific individual high containment laboratory entrance requirements (e.g., vaccinations)
- Service personnel are given opportunity to ask questions and/or consult with the Office of Biosafety

PI or Laboratory Manager

- BSL3 laboratory activities **must stop by 4:00 pm** the day before and decontamination performed as follows:
    - Thorough surface decontamination with an approved disinfectant as outlined in biosafety protocol/BSL3 manual
    - Mop all floors with disinfectant as outlined in biosafety protocol/BSL3 manual
    - After decontamination, no work shall be performed, and no samples or waste is present until maintenance work is complete and service personnel have exited
    - The equipment decontamination verification must be posted on the decontaminated equipment as well as shown to service staff (documentation of decontamination)

    - A room decontamination verification must be posted on the entrance to the decontaminated spaces as well as shown to service staff (documentation of decontamination)
- Ensure all service personnel entering the facility sign the entry/exit log for the BSL3 space
- Laboratory staff are given opportunity to ask questions and/or consult with the Office of Biosafety
- Because service needs may arise during times when high containment activities may not be stopped, we recommend that laboratories create a brief outline for service work during that time. This along with an outline for service work during work stoppage should be added to the Biosafety protocol/BSL3 manual. OBS is willing to assist with the development of a long-term plan.

During Service Work:

Physical Plant/Service Provider

- Can request laboratory personnel move or secure items that could impede service work.
- Service personnel may be asked to adhere to specific individual high containment laboratory requirements
- PPE for the service personnel:
    - Impervious slip resistant (safety) shoes appropriate for ladder safety must be worn. If feasible, fitted shoe covers should worn. If no shoe covers are used, due to safety concerns when using a ladder, shoes and soles must be sprayed and wiped with disinfectant prior to exiting the lab
    - Service personnel should don hand protection (gloves) as frequently as possible. For example, when entering the laboratory and when penetrating surfaces to gain access to equipment. If possible, service personnel should continue to don hand protection at all times unless wearing gloves interferes with mechanical work. Service personnel should not touch laboratory surfaces or laboratory equipment without hand protection.

PI or Laboratory Manager

- Ensure that all potentially infectious materials are safely secured and stored during service activities
- Ensure that service personnel are escorted at all times and made aware of the potential hazards in the laboratory according to the biosafety protocol and BSL3 manual

After Service Work:

Physical Plant/Service Provider

- Thorough handwashing prior to exiting the laboratory
- Equipment/tools: must be surface decontaminated using a disinfectant as outlined in biosafety protocol/BSL3 manual

    - To limit the impact of the disinfectant on the equipment/tools, it is recommended that the equipment/tools also be wiped down with ethanol or water after decontamination

- Sign the entry/exit log, if necessary, for the BSL3 space

PI or Laboratory Manager

- Ensure that service personnel follow proper exit procedures of the laboratory including, signing the entry/exit log, decontaminating tools, and hand washing.

Roles and Responsibilities for Core Facilities

The Office of Biological Safety provides resources and fields questions and/or consults with core and laboratory personnel. The following are recommended for core facilities:

Before Core Work:

Core Personnel

- Provides information on the intake process and any training needed to use equipment and/or enter core facility
- Reviews information on material (source, modifications, antibiotic resistance if applicable, live/fixed, biosafety level, etc.)
- Outlines expectations (e.g., training requirements, PPE, disposal of waste, disinfection/inactivation, emergency response, contact information for core personnel in case of questions or emergencies, etc.)
- Provides other special information as needed
- Provides space or equipment instructions or special information
- May request the biosafety protocol and have the ability to connect with OBS with any questions or concerns
- May refuse materials, services, or entry to lab personnel

PI or Laboratory Personnel

- Provides to the core personnel material description (source, modifications, antibiotic resistance if applicable, live/fixed, biosafety level, etc.)
- Provides contact information to core personnel
- Communicates to the core personnel appropriate method of disinfection/inactivation of materials after experiment is complete
- Communicates to the core personnel appropriate methods for disinfection/inactivation of materials prior to arrival at core facility (e.g., method of fixation, validation of fixation method, etc.)
- Communicates to the core personnel appropriate methods/SOPs for disinfection/inactivation for spills or emergency events
- Communicates to the core personnel occupational health considerations as applicable (e.g., respirator use fit testing, vaccinations)
- Understands outlined expectations (e.g., training requirements, PPE, disposal of waste, disinfection/inactivation, emergency response, contact information for core staff in case of questions or emergencies, etc.)

- Communicates to the core personnel any other special information as needed

During Core Work:

Core Personnel

- Is able to be contacted in the event of an emergency
- Communicates to persons entering if there is a change in entrance requirements (e.g.,, additional personal protective equipment, restricted access) due to other work being performed within the core facility

PI or Laboratory Personnel

- Contacts core staff in emergency situations or when there is a spill
- Adheres to entrance requirements as communicated by the core personnel

After Work is Completed:

Core Personnel

- May check space to ensure readiness for next person

PI or Laboratory Personnel

- Follows proper exit procedures of the laboratory including decontaminating surfaces and/or equipment and hand washing.
- Communicates to the core any other special information as needed
- Completes any follow-up work as needed by the core facility

DURC (DUAL USE RESEARCH OF CONCERN)

The University of Wisconsin-Madison is subject to the United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern (DURC). As a result, the University of Wisconsin-Madison must review all potential dual use research to determine whether or not it meets the criteria outlined in this policy for DURC. Dual use research is research conducted for legitimate purposes that generates knowledge, information, technologies, and/or products that could be utilized for both benevolent and harmful purposes.

Currently this policy covers 15 agents, but the University of Wisconsin-Madison will not limit its review to just these agents. If you work with one of these 15 agents OR you feel your research could potentially be dual use research **fill out the form found on our website:** https://ehs.wisc.edu/labs-research/biological-safety/dual-use-research-of-concern-durc/. For additional information, contact the Institutional Contact for Dual Use Research (ICDUR): https://policy.wisc.edu/library/UW-6094

Avian influenza virus (highly pathogenic)
Bacillus anthracis
Botulinum neurotoxin (all amounts)

Burkholderia mallei
Burkholderia pseudomallei
Ebola virus
Foot-and-mouth disease virus
Francisella tularensis
Marburg virus
Reconstructed 1918 Influenza virus
Rinderpest virus
Toxin-producing strains of *Clostridium botulinum*
Variola major virus
Variola minor virus
Yersinia pestis

RESEARCH WITH SELECT AGENTS

Biological Select Agents or Toxins (BSATs) are biological agents that have been declared by the U.S. Department of Health and Human Services (HHS) or by the U.S. Department of Agriculture (USDA) to have the "potential to pose severe threat to public health and safety." The Federal Select Agent Program regulates the laboratories which may possess, use, or transfer select agents within the United States. To acquire more information on the federal select agent program including a complete list of biological agents and toxins which fall under these regulations go to www.selectagents.gov.

UW- Madison has oversight of research conducted with select agents at UW-Madison. If you are considering starting work with select agents, please contact the UW-Madison Select Agent program Responsible Official/Alternate Responsible Officials (RO/AROs).

The UW-Madison Select Agent Program RO/AROs provide oversight and guidance to the IBC, OBS, UW researchers, and other partners of the program for areas pertaining to research with Select Agents and Toxins performed at UW-Madison. The RO/AROs are charged by the Federal Select Agent Program regulations to have the responsibility to act on behalf of UW-Madison to oversee and manage the security, biocontainment and biosafety, and incident response of select agents and toxins at UW-Madison. As required by the Federal Select Agent Regulations, the RO has been delegated this responsibility by the Vice Chancellor for Research and Graduate Education when s/he is appointed as RO. The AROs have this responsibility when the RO is unavailable.

Subthreshold Select Agent Toxins (Permissible Amounts)

The Federal Select Agent Program does not regulate certain Select Agent (SA) toxins if the amount under the control of a PI does not exceed, at any time, the amounts indicated on their website (https://www.selectagents.gov/permissibletoxinamounts.html). UW-Madison requires that PIs maintain an accurate inventory of SA toxins and secure the toxins in their laboratory. Inventory reports should be submitted semi-annually (every 6 months) to the Office of Biological Safety (biosafety@fpm.wisc.edu). For additional information, contact the Office or Biological Safety.

Permissible amounts are allowed of the following SA Toxins:
Abrin
Botulinum neurotoxins
Short, paralytic alpha conotoxins
Diacetoxyscirpenol (DAS)
Ricin
Saxitoxin
Staphylococcal enterotoxins (Subtypes A, B, C, D, and E)
T-2 toxin
Tetrodotoxin

MINORS

There are inherent risks in allowing minors (i.e., persons less than 18 years of age) to visit, volunteer, or work in research laboratories. In addition to the presence of hazardous biological materials, laboratories may contain chemical and radiological hazards, as well as mechanical, electrical, and thermal hazards from laboratory equipment. Laboratory animals may pose additional risks, including bites, scratches, and exposure to allergens or zoonotic agents. These present risks not only to those performing laboratory work, but also to those who are present within the laboratory while work is ongoing.

Research settings are not limited to laboratories. Farm settings have many physical hazards (e.g., operating farm machinery, handling large animals), and at the agricultural research stations these are coupled with risks from exposure to biological agents (e.g., pathogens in large animal models) and chemicals (e.g., pesticides). Field research may also bring minors into contact with pathogens or allergens in wildlife.

With minors, these risks are greatly amplified because the ability to understand risks and consequences is lower, the effects of accidental exposures can be greater, the skills necessary to handle hazardous materials have not yet been developed, and their response to exposures or other emergencies can be unpredictable. In general, research laboratories and animal facilities are unsuitable for entry by children or by adults with precarious health status. Department chairs/committees, laboratory directors, and PIs should clearly discourage laboratory entry except for scheduled educational activities supervised by an authorized host.

Minors may visit, volunteer, or work in research areas only if the laboratory and activities do not present a high hazard potential. **Some locations, materials, and activities are restricted or prohibited for minors, even if they are matriculated students at UW-Madison.** Please refer to the Minors in Research Laboratories Policy (https://policy.wisc.edu/library/UW-6106) for more information.

RESPONSIBILITIES OF THE INSTITUTIONAL BIOSAFETY COMMITTEE

The Institutional Biosafety Committee (IBC) serves as the Institutional Biosafety Committee required under the *NIH Guidelines*. The committee consists of university and community representatives. University representatives will remain in the majority.

The IBC will support and critically evaluate University of Wisconsin–Madison biological safety activities intended to protect the health and safety of the university community, visitors, and neighbors, and ensure compliance with applicable regulations and guidelines. As part of fulfilling its charge, the IBC will:

- Review protocols that involve biological materials for safety, regulatory compliance, and protection of human health and the environment. Collaborate with other committees, including but not limited to the Animal Care and Use Committees, Radiation Safety Committees, Biosecurity Task Force, and Institutional Review Boards to assure that biological safety issues are properly addressed. Periodically review criteria for mutual referral of protocols.
- Give advice and counsel to the UW-Madison EH&S Office of Biological Safety, the Graduate School, and the Chancellor concerning safe use and management of biological materials and compliance with regulations to support and achieve excellence in biological safety.
- In conjunction with OBS, adopt policies that guide and support the work of OBS and promote high standards of safety, regulatory compliance and protection of human and animal health and the environment in work involving biological materials.
- Review biological safety issues in the OBS publications and on its web site. Perform reviews of campus biological safety programs and biological safety aspects of regulatory compliance documents that require review.
- Review biological safety training programs, records, plans, and priorities as needed to help ensure optimum availability of needed and required training.
- Review proposed regulatory changes and prepare comments to agencies as judged appropriate.
- Provide a forum for the campus community to raise concerns regarding the safe use and handling of biological materials and advise the chancellor in the resolution of disputes regarding biological safety issues.
- Suspend research and/or revoke a protocol in instances where necessary according to the IBC charge and responsibilities.

Administrative support for the functions of the IBC is provided by the UW-Madison Office of Biological Safety.

Approval and registration of the protocols are transmitted to the investigators and to Research and Sponsored Programs in order to satisfy the funding clearance requirements. The direct linkage between review and registration of biosafety protocols and the release of awards is an important aspect of ensuring safe and compliant conduct of research at this institution.

Confidentiality and Conduct

The IBC members shall read, sign, and understand the UW-Madison IBC non-disclosure

agreement (NDA). Failure to sign or abide by this agreement may lead to direct dismissal from the IBC. During meetings subject to state of Wisconsin open meetings law, IBC members shall provide opinions and reviews consistent with the charge of the IBC under the *NIH Guidelines*. Significant deviation from the charge of the IBC may result in direct dismissal from the IBC.

Appointment Process and Length of Service

The IBC members are appointed by the Vice Chancellor for Research and Graduate Education on behalf of the Chancellor at UW-Madison. The BSO provides an annual update of the roster and recommendations to the Associate Vice Chancellor for Research and Graduate Education in Biological Sciences in the OVCRGE. Regular members serve a 3-year term. At the conclusion of the 3-year term, they may elect to continue for an additional 3-year term or to rotate off the committee. The length of service for public members is indeterminate. *Ex officio* members serve as long as they are in their respective positions. The committee chairperson, a faculty member, usually serves in this capacity for at least 1 year.

Appointment Dismissal Procedures

Dismissal from the IBC may occur prior to appointment end for adequate cause related to:

- Deviation from the charge of the IBC
- Breach in confidentiality
- Any other significant cause (e.g., not following or disregarding IBC policies, UW-Madison policies or other governing entity policies, person deemed to be in violation of the *NIH Guidelines* or has had past violations or noncompliance that the person refuses to correct, and actions that threaten or have the potential to threaten UW-Madison accreditations, registrations and/or memberships.)

The IBC chair shall form a dismissal review committee made up of three IBC members plus the chair. The dismissal committee shall review charges and determine if dismissal is appropriate. The IBC chair will communicate, prior to the dismissal committee meeting, to the person in question, and allow a written explanation and/or in person explanation of the cause for dismissal. After a decision is reached by the full IBC, the IBC chair will communicate the decision and any actions required to the person in question. Serving on the IBC is a voluntary position without compensation, and there is no appeals process for any person dismissed by the IBC. The Vice Chancellor for Research and Graduate Education and the Chancellor will be informed by the IBC chair with regards to any dismissal decision.

If the chair is believed to be in violation of any of the above grounds for dismissal, a review committee will instead be formed by the current *ex officio* members of the IBC plus three IBC members of their selection. Dismissal procedures will continue as outlined above, with the exception that the *ex officio* members will be charged with the duties related to dismissal procedure communication normally held by the IBC chair.

Ex officio members of the IBC cannot be dismissed by a review committee, due to the nature of their position. Any and all decisions relating to improper conduct of these members will be handled by their respective office of origin.

Membership of the IBC

The IBC is composed of faculty, a laboratorian, two public members, *ex officio* members, and consultants as per *NIH guidelines* section IV.
Regular members are selected for their expertise in subjects for which the committee will review protocols (see UW-6076). The Chief Campus Veterinarian and Biological Safety Officer (BSO) serve as *ex officio* members. *Ex officio* members may designate an alternate to attend committee meetings and vote in their stead in the event that they are unavailable. Alternates must be approved by the Office of the Vice Chancellor for Research and Graduate Education. Subject matter experts may be appointed as consultants to advise the committee in areas such as human subjects, Select Agents and Toxins, occupational health, and legal affairs.

Protocol Processing

The biosafety protocol serves as a tool to gather relevant information about research that involves the criteria for which a protocol must be submitted. The protocol is submitted using Bio-ARROW (arrow.wisc.edu), and information is available on the OBS web site. There are several different types of submissions:

- **New** protocols are the first submission.
- **Renewals** of existing protocols are required every 3 years.
- **Amendments** must be submitted for changes in research elements, microbes utilized, and/or locations.
- **Grant/Personnel Amendments** serve to change personnel or grant awards to the protocol.

Meeting Procedures and Protocol Review

Reviews of biosafety protocols focus on the risks of the materials and the mitigating measures, and they are very different from grant proposal reviews. The IBC does not judge the merits of the scientific inquiry, traditional ethical considerations (unless it pertains to elements of public safety under the IBC review purview as per *NIH Guidelines*), or review the scientific approach of the research itself, but the committee reviews for the purpose of risk assessment in accordance with the *NIH Guidelines*.

Actions by the IBC on a protocol typically involve one or a combination of the following decisions:

- Approve. The protocol is accepted as provided to the committee.
- Approve with contingency(s). The investigator is required to take additional steps before the protocol will be approved. The protocol must be revised to the satisfaction of the OBS and/or the reviewers.

- Table. The protocol has significant deficiencies that must be addressed before the committee will reconsider it.
- Reject. This action is indicative of significant problems with the protocol. The BSO, and/or reviewers, or designee sends a memo to the investigator explaining the action taken by the IBC.

The following criteria generally are used for selection of full protocols to be reviewed by the IBC:

- Projects involving organisms that could have a significant impact on the environment if accidentally released from the laboratory (e.g., exotic plants, non-indigenous plant pathogens or regulated insects).
- Projects involving activities that are subject to the *NIH Guidelines*, section III-A through III-D
- Projects that require BSL3 containment or involve large scale production under BSL1-LS or BSL2-LS.
- Human gene therapy trials, subject to *NIH Guidelines*, section III-C.
- Select Agents and Toxins
- Protocols involving an issue that OBS is not able to resolve.

Brief summaries of all III-E protocols are reviewed. Any IBC member can call for the full IBC review of a III-E protocol prior to the next meeting. During the meeting, the committee will vote for approval of all posted III-E protocols.

Protocols submitted to OBS that do not require full IBC review (e.g., personnel amendments, non-recombinant DNA protocols, protocols that are exempt from the *NIH Guidelines*, and grant changes) are processed and reviewed by OBS staff.

Previously approved protocols will be submitted for complete review at least every three years. In addition, any changes to the experiment to which the *NIH Guidelines* apply will be reviewed by the IBC. Finally, protocol changes that require significant changes in safety precautions (e.g., changes in PPE, administrative controls, engineering controls) will be reviewed by the IBC.

OBS has the discretion to withhold protocols from the agenda if the protocol is deemed not ready for review.

The IBC may ask the PI or representative to attend the meeting in order to help clarify points and answer questions when their protocol is being reviewed. A PI or representative may attend any open-session IBC meeting or when their protocol is being discussed during closed-session IBC meeting.

Open Meetings Law

The committee is subject to the Wisconsin Open Meetings Law. Actions may be taken only at meetings that are announced and open to the public. A notice of the meeting is

publicly posted. Specific statutory exceptions from the requirement to meet in open session allow the conduct of certain business in closed session.

Closed Session

Protocols may contain information that must be protected due to confidentiality agreements and/or impact of disclosure on competitive positioning or the ability to obtain a patent and/or to ensure the safety and security of research facilities, especially in the case of work involving Select Agents and Toxins or research subject to Dual Use Research of Concern (DURC). Such protocols will be discussed in closed session.

Committee members and consultants are asked to sign a nondisclosure agreement (NDA) when their term begins. Guests who represent particular campus offices are also asked to sign the NDA. Visiting members of the public will not be asked to sign, and they will not be given copies of the protocols. Every protocol is assumed to contain confidential information and release of copies to an individual outside of the committee may be done only with the permission of the PI. If an external person or entity requests a copy of the protocol, the University's records custodian should be contacted so that the request can be handled pursuant to the University's standard processes. Copies of protocols may not be retained by committee members and consultants and must be destroyed (e.g., shredded) prior to disposal.

Meeting Schedule and Location

Meetings of the IBC are tentatively scheduled each month. Unless otherwise noted, IBC meetings usually are held on campus and typically last 1 to 3 hours. Meetings may be cancelled if it is unlikely that a quorum will be present or if there is not enough business to be conducted. Committee members will be notified when this is the case.

RESPONSIBILITIES OF THE OFFICE OF BIOLOGICAL SAFETY

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

- Advises faculty and staff in biosafety matters.
- Provides guidance on recombinant DNA (rDNA) regulations or other aspects of genetic engineering.
- Recommends safe procedures, containment devices, and equipment for all campus activities (research, teaching, diagnostic, and building services) involving biohazards.

- Recommends methods of handling, transporting, decontaminating, and disposing of biohazardous materials.
- Provides advice regarding the disposal of biohazardous waste and biological toxins.
- Provides consultation for containment laboratory/ventilation system design.
- Provides consultation concerning the purchase of biological safety cabinets (BSCs)
- Provides biological safety education and training aids; develops educational and training programs designed to meet the specific biological safety needs of a variety of departments and staff.
- Provides a variety of biological safety references, resources, and guidance materials online or in the Office of Biological Safety
- Provides biohazard signs, BSL signs, and emergency door cards.
- Provides training and certification for compliance with U.S. Department of Transportation and international regulations for shipping hazardous biological materials.
- Provides administrative support to the Institutional Biosafety Committee (IBC)
- Take notes during the meeting to develop an accurate record of the deliberations.
- The BSO facilitates the discussion, as needed.
- Prepare the minutes of the meeting.
- Provide information to PIs explaining the committee's action on their protocol.
- Follow up on any action requested by the committee.
- Draft policies for the committee's consideration, as appropriate.
- Report incidents, such as significant laboratory accidents and laboratory acquired infections, and violations of the *NIH Guidelines* and institutional policies.
- Provide information to the IBC as relevant issues come to light.
- Keep the IBC apprised of regulatory and scientific developments that pertain to biosafety.

OBS staff performs a review of all biosafety protocols. The Principal Investigator (PI) as well as person(s) listed under "Read/Write Personnel" are contacted if additional information or clarification is needed for completion of the risk assessment. Protocols must be submitted well in advance of the IBC meeting, typically by the previous meeting, to allow time to address outstanding issues. Protocols that require review by the IBC usually cannot be expedited.

Protocols are valid for 3 years and must be kept up to date during that period through submission of amendments, as applicable.

The final step in processing protocols is to issue the registration form. Once the final version of a protocol (new, renewal, or amendment) has been reviewed and approved by OBS (e.g., OBS ensures IBC contingencies are met, as applicable), the registration form is generated in Bio-ARROW and the research team is notified by email.

Biosafety Administration

- **Federal Guidelines:** Certain research is subject to federal guidelines and regulations prescribed by the NIH, CDC, the U.S. Department of Agriculture (USDA), the U.S. Environmental Protection Agency, and the U.S. Food and Drug Administration.
  Investigators utilizing human blood and other potentially infectious human materials must meet certain requirements. The Occupational Health Officer can assist you in this area.
- **State Law Regarding rDNA Field Studies:** The State of Wisconsin has enacted a law requiring that the Wisconsin Department of Natural Resources or Department of Agriculture, Trade and Consumer Protection (DATCP) be notified of intended field studies of genetically engineered organisms.
- **Wisconsin Department of Natural Resources Guidelines for Waste Disposal:** The DNR has established regulations for the decontamination and elimination of infectious and medical wastes. Appropriate disposal of these wastes is an important aspect of a comprehensive safety program. WI Administrative Codes Chapter NR 526 Medical Waste Management, 2006.
- **Wisconsin Department of Commerce Regulations/OSHA Bloodborne Pathogens Standard:** As a public institution, the university must also comply with regulations prescribed by the Wisconsin Department of Commerce, including the Bloodborne Pathogens Standard mandated by the Occupational Safety and Health Administration (OSHA).

Biosafety Training

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

Individuals should maintain a record of training activities (Training Record), including not just formal classroom sessions but also topics covered during staff meetings and one-on-one mentoring.

Required Training

The OBS offers biological safety training courses designed to inform and prepare you for work in campus biological research laboratories in compliance with standards set forth by the NIH and CDC. Courses are offered online through Learn@UW.

The Biosafety Required Training course is required training for any person working in a biological laboratory in accordance with a registered biosafety protocol and must be renewed every 5 years. Individuals who completed the previous required training courses (Biosafety 101, 104, and 201) will need to complete the Biosafety Required Training course when training expires for Biosafety 101, Biosafety 104, or Biosafety 201.

Biosafety 102 is required for any laboratory staff working with human-derived materials and must be renewed annually.

All other Biosafety trainings are not required by OBS but may be required by individual laboratories, PIs, Lab Managers or departments.

To register for any Biosafety course, please go to our website training pages and select the desired course to locate registration information. All courses are self-registration and ongoing, but you must register through our website https://ehs.wisc.edu/biosafety-training/.

Biosafety Required Training Course
Required for all personnel working in laboratories with biohazardous materials when the work is covered by a Biosafety Protocol.
Renew training every 5 years.

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

Renew training every year.

Biosafety 105: Biosafety Cabinet Use
Guidance training for any personnel working in laboratories using Biological Safety Cabinets (BSCs).

Biosafety 106: Autoclave Use
Guidance training for any personnel working in laboratories using autoclaves.

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

Biological Materials and Dry Ice Shipping Training:

Biosafety 205: Bio-HazMat Shipping Training
This Learn@UW Canvas course provides initial training and renewal training for hazardous materials shipping of biological materials and dry ice compliant with IATA/ICAO Dangerous Goods and US DOT 49CFR Hazardous Materials Regulations. Once registered, this course will be continually available to you for any renewal or refresher training or to use reference materials within the course.

This course consists of 6 Training Pathways, each earning a certificate of training. Each Path is independent with its own quiz and certificate; you only complete the paths you need for the materials you intend to ship. Different materials have different certification

and training expiration/requirements which are described in the course paths and on the quiz/certificate printouts.

To complete a training path you must have a passing score on the quiz and print the quiz score page for your certificate. Each quiz results page should be retained as the training certificate for your records and will display your name, date completed, quiz score, description of training and regulation references.

Biosafety 205 Training Paths

- Campus Transport of Biologicals and Dry Ice Path *(no expiration)*
- Exempt & Unregulated Biological Shipping Path *(no expiration)*
- GMO/GMMO Shipping Path *(must renew training every 24 months)*
- Biological Substance, Category B Shipping Path *(must renew training every 24 months)*
- Infectious Substance Category A Shipping Path *(must renew training every 24 months)*
- Dry Ice Shipping Path *(must renew training every 24 months)*

Please Note:

- We will no longer email certificates.
- We do not send reminders to renew shipping training. Please set a reminder to renew your training according to the expiration/renewal described in the training path(s) you've completed.
- This training is not tracked in BioARROW.
- The certificate issued by EH&S Office of Biological Safety (OBS) certifies completion of compliant training on a specific date by the employee; your individual employer (e.g. department, group, laboratory, manager, PI, etc.) may accept this training as adequate for your designation as a certified hazmat employee.
- Federal law requires training in the regulations so your employer can certify you to ship hazmat. You and your employing department or laboratory group may choose compliant shipping training outside of UW and you are not obligated to use this specific course, so long as you complete compliant training to ship materials.

DURC: Dual Use Research of Concern

Dual Use Research of Concern (DURC) training is required for personnel listed on a biosafety protocol that includes research activities with any of the 15 agents covered by the DURC policy.
Renew training every 3 years.

Biosafety Training Materials for Select Agent Labs

Individuals in the Select Agent Program will be notified of required training and receive access by invitation only. Any questions regarding the types and frequency of required

training can be directed the Responsible Official or Alternate Responsible Officials for the UW-Madison Select Agent Program.

Additional Training
Additional trainings are presented by OBS trainers and staff on request. Our trainings may cover specialized topics, discussions or address specific audiences (e.g., first year graduate student biosafety orientation, summer science interns, emergency lab drills).

Laboratory Visits

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

- Meet the needs of researchers for guidance on biosafety and regulatory issues.
- Facilitate communication between staff and OBS.
- Discuss facility issues.
- Ensure that our records accurately reflect ongoing research activities.

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

USEFUL REFERENCES

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

American Biological Safety Association (ABSA) list of risk groups: https://my.absa.org/Riskgroups

Arthropod Containment Guidelines. Version 3.2 (2019), A project of the American Committee of Medical entomology of the American Society of Tropical Medicine and Hygiene: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6396570/

Biosafety in Microbiological and Biomedical Laboratories, CDC/NIH. Current Edition: https://www.cdc.gov/labs/BMBL.html

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

National Sanitation Foundation Standard (NSF) 49, Biological Safety Cabinets, 2002:
http://www.nsf.org/business/biosafety_cabinetry/index.asp

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules:
https://osp.od.nih.gov/biotechnology/nih-guidelines/

NTP Report on Carcinogens. National Toxicology Program, Department of Health and Human Services:
https://ntp.niehs.nih.gov/whatwestudy/assessments/cancer/roc/index.html

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):
http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=standards&p_id=10106

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, Current Edition: https://www.cdc.gov/labs/BMBL.html

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:
https://vtechworks.lib.vt.edu/handle/10919/78423

World Health Organization (WHO) Laboratory Biosafety Manual. 3rd Ed. revised. Geneva, 2004:
http://www.who.int/csr/delibepidemics/WHO_CDS_CSR_LYO_2004_11/en/