

Biosafety Manual



Oklahoma State University
University Research Compliance - Biosafety Office
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INTRODUCTION

DEFINITION OF BIOHAZARDOUS MATERIAL

Biohazardous material includes all viable infectious, pathogenic, or toxin producing agents, prions, biologically derived toxins, or nucleic acid constructs that have the potential to affect the health of humans, animals, plants, or the environment (FAQs Biosafety Terms, n.d.). This also includes vectors known to carry and transmit infectious agents and infected or potentially infected animals (George Mason University, 2022).

PURPOSE & SCOPE

The Oklahoma State University (OSU) Biosafety Office has developed this manual to provide information regarding appropriate practices, University policies, and regulatory requirements for working safely with biohazardous materials. It is intended to enable and encourage those working with biohazardous materials to work safely and to reduce or mitigate any risk associated with the work.

The procedures specified herein are applicable to all research and instructional activities involving biohazardous materials on the OSU-Stillwater and OSU-Tulsa campuses. The practices and procedures presented in this manual are based on those detailed in:

- [National Institutes of Health \(NIH\) Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules](#) (NIH Guidelines); and
- [Biosafety in Microbiological and Biomedical Laboratories](#) (BMBL).

This manual does not address radiation or chemical safety. These areas are covered in the OSU Radiation Safety Handbook, which is the purview of the Radiation Safety Committee, and the OSU Chemical Hygiene Plan, which is the purview of Environmental Health and Safety (EHS). For researchers working with select agents and/or toxins, an additional operations and procedures manual is provided (i.e., *Biological Research Safety Plan for Select Agent & Toxin Use*).

ROLES & RESPONSIBILITIES

Vice President for Research

The Vice President for Research (VPR) leads the effort to ensure that all research and teaching activities involving the use of biohazardous materials, and the facilities used to conduct such work, are in compliance with all external regulations and applicable University policies.

Institutional Biosafety Committee

The President of OSU has conferred upon the VPR the authority to appoint an Institutional Biosafety Committee (IBC). The IBC has responsibility for review and approval of research protocols and procedures related to biosafety and for regular inspection of research laboratories and facilities that fall within its purview. Additional responsibilities of the IBC can be found in the OSU Institutional Biosafety Policy (4-0301).

University Research Compliance

Biosafety personnel within the Office of University Research Compliance (URC), working in concert with the IBC, are responsible for development and oversight of procedures and practices for safe handling of biohazardous materials at OSU. This office is also responsible for ensuring compliance with the *NIH Guidelines* and all other applicable biosafety guidelines and policies.

Supervisors

The principal investigator (PI), instructor, or supervisor must ensure compliance with all Federal, State, and OSU policies and procedures regarding biosafety. He/she is also responsible for the safe operation of his or her laboratory.

Personnel & Students

Individuals who work with biohazardous materials must adhere to all applicable biosafety guidelines and policies and shall consult with their supervisors regarding handling and disposal of specific biohazardous materials that they use.

Immunocompromised individuals are responsible for ensuring that they are able to safely work in the laboratory. These individuals should consult with their supervisor and a physician regarding potential risks and ways in which those risks can be managed. The following may make individuals more susceptible to infection: disease, other medical conditions, or drugs that alter host defense; allergenic hypersensitivity; and inability to receive specific vaccinations. Skin diseases such as chronic dermatitis, eczema, and psoriasis can create breaks in the skin that may allow pathogen entry. Antibiotic or antimicrobial treatment may change the composition of the natural microbial flora of the mucous membranes or digestive system, leaving the individual more susceptible to colonization by infectious microorganisms. Other conditions and treatments such as diabetes, cancer chemotherapy, steroid treatments, or HIV infection may also cause immunodeficiencies. Women who are pregnant are also considered to be immunocompromised.

PROJECT ASSESSMENT AND APPROVAL

RISK ASSESSMENT

Biosafety Levels

The biosafety level (BSL) is a description of the degree of physical containment being employed to confine biohazardous material within a laboratory or facility and to reduce the potential for exposure of laboratory workers, persons outside of the laboratory, and the environment. Each BSL consists of a combination of laboratory practices and techniques, safety equipment, and laboratory facilities which are approved for research involving specific materials. The *BMBL* provides detailed criteria for laboratory biosafety levels (BSL-1 through BSL-4) and animal biosafety levels (ABSL-1 through ABSL-4). OSU researchers currently conduct work at the following biosafety levels: BSL-1, BSL-2, BSL-3, ABSL-1, ABSL-2, and ABSL-3. Each of these biosafety levels is summarized below.

Biosafety Level 1 (BSL-1)

BSL-1 is suited for activities involving agents that are not known to cause disease in healthy adult humans and that present minimal hazards to lab personnel and the environment. Work is conducted on open lab benches while utilizing standard microbiological practices. Special containment equipment and facility design is not typically required. Laboratory personnel receive training on laboratory procedures.

Biosafety Level 2 (BSL-2)

BSL-2 is suited for activities involving agents that are moderately hazardous to lab personnel and/or the environment. Access to the laboratory is restricted and lab personnel are trained on handling of pathogenic agents. All procedures with the potential to create infectious aerosols or splashes are conducted in a biological safety cabinet (BSC) or other containment device.

Biosafety Level 3 (BSL-3)

BSL-3 is suited for activities involving indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure. The lab has special engineering and design features and all laboratory personnel receive specific training on handling potentially lethal agents. All procedures involving the manipulation of infectious agents are conducted within a BSC or other containment device.

Animal Biosafety Level 1 (ABSL-1)

ABSL-1 is suited for animal work involving agents that are not known to cause disease in healthy adult humans or other animals and that present minimal hazards to personnel and/or the environment. Special containment equipment or facility design may be required as determined by risk assessment. Personnel receive specific training in facility procedures.

Animal Biosafety Level 2 (ABSL-2)

ABSL-2 is suited for animal work involving agents that are associated with human or animal disease and that present moderate hazards to personnel and the environment. This level addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure. Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment.

Animal Biosafety Level 3 (ABSL-3)

ABSL-3 is suited for animal work involving indigenous or exotic agents, agents that present a potential for aerosol transmission, and agents causing serious or potentially lethal disease in humans or animals. The ABSL-3 laboratory has special engineering and design features. Additionally, procedures involving the manipulation of infectious materials, or where aerosols or splashes may be created, must be conducted in a BSC or other containment device. Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment. Employee occupational health programs must be implemented.

Plant Containment Levels

The plant containment level is a description of the degree of containment being employed to prevent the transmission of a recombinant or synthetic nucleic acid [r(s)NA] molecule-containing plant genome or the release of r(s)NA-derived organisms (i.e., microorganisms and/or arthropods) associated with plants during experiments conducted in a greenhouse. Each containment level consists of a combination of greenhouse practices and special greenhouse facilities for physical containment. The *NIH Guidelines* provide detailed criteria for plant biosafety levels (BSL-1P through BSL4-P). OSU researchers currently conduct work at BSL-1P and BSL-2P. Each of these containment levels is summarized below.

Biosafety Level 1 – Plants (BSL-1P)

BSL-1P is suited for greenhouse activities involving transgenic plants that would not be able to survive or spread in the environment or that would not otherwise pose an environmental risk if accidentally released. BSL-1P also applies to genetically modified plant-associated organisms that are not known to have negative effects on the ecosystem and do not spread rapidly (Adair

& Irwin, 2008). Experimental organisms must be kept in species-appropriate containers and must be rendered inactive prior to disposal. Special facility design is not required.

Biosafety Level 2 – Plants (BSL-2P)

BSL-2P is suited for activities with transgenic plants and associated organisms that could be easily managed if released outside of the greenhouse and for transgenic plants that may display new weedy characteristics or are capable of interbreeding with weeds in the area. This containment level is also suited for transgenic plant-associated organisms that do not pose a serious threat to the ecosystem (Adair & Irwin, 2008). In addition to BSL-1P requirements, work at this level warrants additional signage and documentation. Additionally, the greenhouse floor must be impervious and openings (e.g., windows, roofs, etc.) must be screened to exclude small flying animals.

Arthropod Containment Levels

The arthropod containment level (ACL) is a description of the degree of confinement being employed to contain arthropod vectors of pathogenic agents. When an arthropod is infected with a pathogen, the containment level must match that required for the agent without regard for other factors (e.g., vector competence, etc.). Each ACL consists of a combination of laboratory practices and primary and/or secondary barriers. The [Arthropod Containment Guidelines](#) provide detailed criteria for arthropod containment levels (ACL-1 through ACL-4). OSU researchers currently conduct work at ACL-1 and ACL-2. Each of these arthropod containment levels is summarized below.

Arthropod Containment Level 1 (ACL-1)

ACL-1 is suited for activities involving uninfected arthropod vectors or vectors that are infected with a non-pathogen. Arthropods that are already present in the region and exotic species that would be inviable upon escape are typically maintained under ACL-1 conditions. Arthropods are handled using standard practices and housed in species-appropriate containers.

Arthropod Containment Level 2 (ACL-2)

ACL-2 is suited for work with exotic and indigenous arthropods that are infected with human and/or animal pathogens. Genetically modified vectors should also be maintained at this level as long as the modifications have no, or only negative effects on survivorship, viability, or vector capacity. Arthropods are still housed in species-appropriate containers. However, requirements for facility access, physical containment, disposal, and facility design are more stringent than those for ACL-1.

Risk Groups

Biological agents and toxins may be classified into risk groups based on their relative hazard. The table that follows describes the classification of biological agents and toxins based on the risk to both humans and animals.

Risk Group Classification	NIH Guidelines for Research Involving Recombinant DNA Molecules 2019	World Health Organization Laboratory Biosafety Manual 4th Edition 2020
Risk Group 1	Agents that are not associated with disease in healthy adult humans.	(No or low individual and community risk) A microorganism that is unlikely to cause human or animal disease.
Risk Group 2	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are <i>often</i> available.	(Moderate individual risk; low community risk) A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.
Risk Group 3	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk)	(High individual risk; low community risk) A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.
Risk Group 4	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are <i>not usually</i> available (high individual risk and high community risk)	(High individual and community risk) A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

The *BMBL* and *NIH Guidelines* do not contain definitions for risk group classification of biological agents and toxins that pose a risk to plants. However, both define biosafety levels to contain agents that pose a threat to the environment. To determine a risk group classification for biological agents and toxins that pose a risk to plants, *A Practical Guide to Containment: Plant Biosafety in Research Greenhouses* may be referenced.

Risk groups and containment levels often correspond. However, the IBC may elect to raise or lower the biosafety level for work with a particular agent based upon risk assessment.

Agent Specific Risk Assessment

It is the responsibility of the PI or laboratory director to conduct a risk assessment to determine the proper work practices and containment requirements for work with biohazardous material. The risk assessment process should identify features of microorganisms as well as host and

environmental factors that influence the potential for workers to experience a biohazard exposure. To document the risk assessment, the appropriate IBC protocol and risk assessment forms must be completed. This responsibility cannot be shifted to inexperienced or untrained personnel. The IBC must approve the risk assessment before work may be conducted.

The PI or laboratory director should consult with the Biosafety Officer (BSO) to ensure that the laboratory is in compliance with established guidelines and regulations. When performing a risk assessment, it is advisable to take a conservative approach when the available information is incomplete. Factors to consider when evaluating risk include:

Host range: Note the susceptible hosts and whether susceptible hosts are in the vicinity of the proposed research.

Disease severity: Consider the symptoms that the agent or toxin causes. The potential to survive infection with and without medical treatment should also be described. Note that different strains of an agent may impact disease severity.

Route of transmission: Note how the agent causes infection (e.g., inhalation, ingestion, breaks in the skin, etc.). If a vector is needed for transmission, describe this relationship. Also note if infection can be spread from person to person, animal to animal, or plant to plant.

Agent stability: The greater the potential for an agent to survive in the environment, the higher the risk. Consider factors such as desiccation, exposure to sunlight or ultraviolet light, or exposure to chemical disinfectants when looking at the stability of an agent. It is important to note the agent's ability to survive both on a surface in the lab and if it accidentally released into the environment.

Form of agent: Note which developmental stages of the agent you are using if the agent has different forms (e.g., spores, vegetative cells, etc.).

Infectious dose: Consider the amount of an infectious agent needed to cause infection in a normal host. An infectious dose can vary from one to hundreds to thousands of organisms or infectious units. A host's immune status can also influence the infectious dose.

Concentration and volume: Consider whether the organisms are in solid tissue, viscous blood, sputum, urine, feces, etc., the volume of the material, and the laboratory work planned (e.g., amplification of the material, sonication, centrifugation, etc.). In most instances, the risk increases as the concentration of microorganisms increases.

Origin: This may refer to the geographic location (domestic or foreign), host, or nature of the source.

Availability of data from animal studies: If human data is not available, information on the pathogenicity, infectivity, and route of exposure from animal studies may be valuable. Use caution when translating infectivity data from one species to another.

Drug resistance: Describe any drug resistance and note if the drug is commonly used to treat the disease and agent causes.

Availability of effective prophylaxis or therapeutic intervention: Effective vaccines, if available, should be offered to laboratory personnel in advance of their handling of infectious material. However, immunization does not replace engineering controls, proper practices and procedures, and the use of personal protective equipment (PPE). The availability of post-exposure prophylaxis should also be considered.

Medical surveillance: Medical surveillance programs may include monitoring employee health status, participating in post-exposure management, employee counseling prior to offering vaccination, and annual checkups.

PROJECT APPROVAL

All projects involving biohazardous materials must be approved by the IBC prior to initiation. Aspects of certain projects may also require the approval of the NIH Director and/or NIH's Office of Biotechnology Activities (OBA), the Institutional Animal Care and Use Committee (IACUC), the Radiation Safety Committee, the Laser Safety Committee, the Institutional Review Board (IRB), and Environmental Health and Safety (EHS), as well as others.

IBC

All research and teaching activities conducted by faculty, staff, students, post docs, visiting scientists or other temporary personnel on OSU property or involving the use of OSU-owned equipment are subject to IBC review if the activities involve the use of biohazardous materials as defined in this manual. Important information regarding the submission of an IBC protocol application follows:

- All applicable sections must be completed, all signatures and initials obtained, and all required documentation must be provided to the IBC prior to its review of a protocol.

- The project summary must be easily understood by a diverse group of people, including individuals without expertise in the specific field. At the same time, it must provide enough detail for the committee to evaluate the work for the purpose of performing a risk assessment. Insufficient information will make it difficult for the IBC to assess the potential hazards and risks of the work which will result in approval delay. The following information should be included in the summary:
 - overall goals and significance of the work;
 - specific objectives/phases;
 - experimental procedures to be used;
 - PPE, safety equipment, waste processing, disinfection procedures, transport procedures, sharps handling, and any other lab safety procedures; and
 - specific locations for various steps if the research is to be conducted in multiple locations.

After submission of the complete protocol, the IBC will review it to determine if the proposed project is in compliance with the appropriate policies and regulations. IBC review will consist of, but is not limited to:

- an overall assessment of the proposed project to determine if any conditions associated with the project would prohibit initiation of the proposed plan;
- an assessment of the containment level proposed to ensure that the level is sufficient for the type of activity being proposed; and
- an assessment of the facilities, procedures, practices, and training relative to the proposed level of containment.

The IBC, as part of its review of an application, will ensure that a biosafety inspection report for the particular space(s) listed in the application is current and any noted deficiencies from that inspection have been properly addressed.

No research employing biohazardous materials can commence prior to IBC approval, regardless of source of funding (if any). Any modification of the original protocol will require the approval of the Biological Safety Officer or the IBC depending upon the nature of the modification.

Examples of project changes that require IBC approval include:

- change in scope of work;
- change in procedures or equipment;
- change in agents;
- change of strains;

- change in drug resistance of strains;
- change in laboratory space; and/or
- change in personnel.

NIH Director & NIH-OBA

In addition to IBC approval, some experiments involving recombinant or synthetic nucleic acids must also be approved by the NIH Director or by NIH-OBA. Major Actions, as defined by the *NIH Guidelines*, must be approved by the NIH Director. Experiments involving the cloning of toxin molecules with an LD50 of less than 100 ng/kg body weight must be approved by NIH-OBA.

IACUC

All research involving live, vertebrate animals must be approved by the OSU IACUC prior to initiation.

Radiation Safety

All research involving radioactive materials and/or machines which produce ionizing radiation must be approved by the OSU Radiation Safety Committee prior to initiation.

Laser Safety

All research involving Class 3B and/or Class 4 lasers must be approved by the OSU Laser Safety Committee prior to initiation.

IRB

All research involving human subjects must be approved by the OSU IRB prior to initiation. In addition, if the research involves the deliberate transfer of recombinant or synthetic nucleic acids, the Recombinant Advisory Committee (RAC) of the NIH-OBA must also review and approve the project.

EHS

Blood, blood products, body fluids, and/or tissues from humans or non-human primates: The Occupational Safety & Health Administration (OSHA) Bloodborne Pathogen Standard mandates a combination of engineering and work practice controls, training, Hepatitis B vaccination, and other provision to help control the health risk posed to employees resulting from occupational exposure to human blood and other potentially infectious materials that may contain these or other specified agents.

EHS is responsible for facilitating compliance with OSHA standards, including the Bloodborne Pathogen Standard. Contact EHS for additional requirements for working with these materials.

Respiratory Protection Program: The purpose of the Respiratory Protection Program is to ensure that all OSU employees have adequate respiratory protection in the workplace when engineering controls or work practices are inadequate or not feasible to reduce the exposure to airborne contaminants.

EHS provides respiratory protection services to all OSU-Stillwater employees who wear respiratory protection during work activities and those who anticipate wearing respiratory equipment during an emergency incident. Contact EHS for additional information on this program.

PERMITS

Special federal permits may be required for importing, exporting, and/or transporting human pathogens, animal pathogens, animals or animal products, plant pathogens or plant pests, and plants or plant products. PIs are responsible for obtaining all required permits prior to the initiation of research involving regulated materials. Permit requirements should be verified well in advance of needing the material in question as some permits can take up to 18 months to process.

Pathogens & Pests of Agricultural Significance

The United States Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) regulates the transport of materials that have the potential to harm U.S. agricultural products, such as livestock or crops. For this reason, APHIS permits may be required for import, export, and/or movement of animal or plant pathogens, soil samples, insects, animals or animal products, and plants or plant products. An APHIS permit may also be required for introduction of genetically modified organisms into the environment. Information about the APHIS permits that are commonly required for academic research can be found below. Additional information can be found on the USDA-APHIS Imports & Exports [webpage](#).

Plant Protection and Quarantine (PPQ) Permits: APHIS PPQ regulates the movement of agricultural and associated products to safeguard U.S. agriculture and natural resources from the risks associated with the entry, establishment, or spread of plant pests and noxious weeds.

- Regulated Organism Permits – A PPQ 526 permit is required for the importation, interstate movement, and environmental release of plant pests (plant feeding insects, mites, snails, slugs, and plant pathogenic bacteria, viruses, fungi, etc.), biological control organisms of plant pests, and weeds, bees, parasitic plants, and noxious weeds. A PPQ 526 permit is also required for importation and interstate movement of soil for the purpose of isolating or culturing microorganisms from the soil.

A facility inspection may be required before a PPQ 526 permit can be issued. Applicants will be informed of the need for an inspection once submitted application materials have been reviewed by a PPQ specialist.

Note: A PPQ 526 permit is required anytime that you are importing one of the materials listed above into Oklahoma, even if the organism is already present in the state.

- Soil Permits – A PPQ 525 permit is required for the importation of soil for purposes other than the isolation and culturing of microorganisms from the soil. This permit is required when importing soil for chemical/physical analysis and for isolation of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA).

Veterinary Services (VS) Permits: APHIS VS aims to control, prevent, and/or eliminate animal diseases by monitoring animal health in the U.S. VS provides permits for the import, interstate movement, and export of materials exposed to or derived from animals. VS offers the following permits.

- APHIS 2005 – Application for U.S. Veterinary Biological Product Permit
- VS 16-3 – Application for Permit to Import Controlled Material or Transport Organisms or Vectors or Animal Products and By-Products
- VS 16-7 – Application for Permit to Import Cell Cultures and Their Products
- VS 17-129 – Application to import or transit live animals, semen, embryos and eggs for hatching

A facility inspection may be required before a VS permit is issued.

Biotechnology Regulatory Service (BRS) Permits: BRS issues permits for importation, interstate movement, or environmental release of certain genetically engineered organisms that pose a plant pest risk, including plants, insects, or microbes. The introduction of regulated articles requires a permit with the exception of introductions that are either eligible for introduction under the notification procedure or are conditionally exempt.

Regulated Article - an organism which has been altered or produced through genetic engineering, if the donor organism, recipient organism, or vector or vector agent belongs to any genera to taxa designed in [7 CFR 340.2](#) and meets the definition of plant pest, or is an unclassified organism and/or an organism whose classification is unknown, or any product which contains such an organism, or any other organism or product altered or produced through genetic engineering which the APHIS Administrator determines is a plant pest or has reason to believe is a plant pest

- Notification Procedure – The notification procedure is a streamlined alternative to the permitting process which allows the introduction of a certain subset of genetically engineered plants. To qualify for the notification process, the applicant must certify that: 1) the regulated article meets specific eligibility criteria, and 2) the introduction will meet specified performance standards. Detailed information about these eligibility criteria and performance standards can be found in the USDA-APHIS BRS [Notification User Guide](#).
- Exemptions –
 1. A permit for interstate movement is not required for genetic material from any plant pest in *Escherichia coli* genotype K-12 (strain K-12 and its derivatives), sterile strains of *Saccharomyces cerevisiae*, or asporogenic strains of *Bacillus subtilis*, provided that the following conditions are met:
 - a) the microorganisms are packaged and shipped in accordance with applicable APHIS [guidelines](#);
 - b) the cloned genetic material is maintained on a nonconjugation proficient plasmid and the host does not contain other conjugation proficient plasmids or generalized transducing phages;
 - c) the cloned material does not include the complete infectious genome of a known plant pest; and
 - d) the cloned genes are not carried on an expression vector if the cloned genes code for:
 - a toxin to plant or plant products, or a toxin to organisms beneficial to plants; or
 - other factors directly involved in eliciting plant disease (i.e., cell wall degrading enzymes); or
 - substances acting as, or inhibitory to, plant growth regulators.
 2. A permit for interstate movement is not required for genetic material from any plant pest contained in the genome of the plant *Arabidopsis thaliana*, provided that the following conditions are met:
 - a) the plants or plant material are packaged and shipped in accordance with applicable APHIS [guidelines](#);
 - b) the cloned genetic material is stably integrated into the plant genome; and

- c) the cloned material does not include the complete infectious genome of a known plant pest.

Human Pathogens

The U.S. Department of Health and Human Services (HHS), through the Centers for Disease Control and Prevention (CDC), regulates the importation of infectious biological agents, infectious substances, and vectors of human disease into the U.S. These regulated materials are listed below.

- Infectious biological agents – a microorganism (including, but not limited to, bacteria, viruses, fungi, or protozoa) or prion, whether naturally occurring, bioengineered, or artificial, or a component of such microorganism or prion that is capable of causing communicable disease in a human
- Vectors – any animal (vertebrate or invertebrate) including arthropods or any noninfectious self-replicating system (e.g., plasmids or other molecular vector) or animal product (e.g., a mount, rug, or other display item composed of the hide, hair, skull, teeth, bones, or claws of an animal) that are known to transfer or are capable of transferring an infectious biological agent to a human
- Animals – any member of the animal kingdom except a human including an animal product (e.g., a mount, rug, or other display item composed of the hide, hair, skull, teeth, bones, or claws)
- Arthropods – any living insect including crustaceans, spiders, scorpions, etc. capable of being a host or vector of human disease
- Snails – any freshwater (phylum Mollusca, class Gastropoda) snail capable of transmitting schistosomiasis
- Bats – all live bats
- Non-human primate material – all non-human primate material (e.g., blood, plasma, tissue, urine, feces) requires an import permit, unless it has been specifically treated and rendered non-infectious

Personnel from the CDC may inspect applicants to ensure that the facilities have implemented the appropriate biosafety measures for the infectious biological agents, infectious substance, or vector to be imported.

Additional information can be found on the CDC Import Permit Program (IPP) webpage at: <http://www.cdc.gov/od/eaipp/>.

BIOSAFETY CONTAINMENT, PRACTICES, & PROCEDURES

CONTAINMENT

The term “containment” is used to describe the safe methods for managing infectious agents in the laboratory environment where they are being handled or stored. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other people, and the outside environment to potentially hazardous agents. The three elements of containment are: 1) laboratory practice and technique, 2) safety equipment, and 3) facility design.

Primary Containment

Protection of personnel and the immediate laboratory environment from exposure to infectious agents is considered primary containment. It is attained by good microbiological technique and the use of appropriate safety equipment.

- **Primary Containment Equipment:**
Safety equipment includes Class II and III BSCs, HEPA-filtered fermenters, enclosed containment (e.g., safety centrifuge cups) and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The BSC is the principal device used to provide containment of infectious splashes or aerosols generated by many microbiological procedures.

Safety equipment may also include PPE such as protective clothing, respirators, face shield, and safety glasses or goggles. PPE is often used in combination with other safety equipment when working with biohazardous materials. In some situations, personal protective clothing may form the primary barrier between personnel and the infectious materials.

In some cases, the laboratory itself may be considered primary containment (e.g., a laboratory housing animals exposed to infectious agents in open cages).

Secondary Containment

The protection of the environment external to the laboratory from exposure to infectious materials is considered secondary containment. It is attained by a combination of facility design and operational practices. The risk assessment of the work to be done with a specific agent will determine the appropriate combination of work practices, safety equipment, and facility design to provide adequate containment.

- **Facility Design:**
The design of a facility is important in providing a barrier to protect people working inside and outside the laboratory, and people, plants, and animals in the community

from infectious agents by reducing the probability of them being released from the laboratory. Facilities must be commensurate with the laboratory's function and the recommended biosafety level for the agent being manipulated.

The recommended secondary barrier(s) will depend on the risk of transmission of specific agents. For example, the exposure risks for most laboratory work in Biosafety Level 1 and 2 facilities will be direct contact with the agents, or inadvertent contact exposures through contaminated work environments. Secondary barriers in these laboratories may include separation of the laboratory work area from public access, availability of a decontamination facility (e.g., autoclave), and hand washing facilities.

As the risk for aerosol transmission increases, higher levels of primary containment and multiple secondary barriers may become necessary to prevent infectious agents from escaping into the environment. Such design features could include specialized ventilation systems to assure directional airflow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks at laboratory entrances, or separate buildings or modules for isolation of the laboratory.

Laboratory Practice and Technique

The most important element of containment is strict adherence to standard microbiological practices and techniques. Individuals working with infectious agents or infected materials must be aware of potential hazards, and must be trained and proficient in the practices and techniques required for handling such material safely. The PI or laboratory supervisor is responsible for providing or arranging for appropriate training of personnel.

The following standard microbiological practices must be adhered to at all times:

- Lab personnel must wash their hands after handling biohazardous materials, removing gloves, or leaving the containment area.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas.
- Mouth pipetting is prohibited; mechanical pipetting devices must be used.
- Perform all procedures in a way that minimizes the creation of splashes and/or aerosols.
- Decontaminate work surfaces after completion of work with biohazardous materials and after any spill or splash of potentially infectious material with appropriate disinfectant.
- Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method.

- An effective integrated pest management program is required (see Appendix E for details).
- The PI or laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures.

Each laboratory must develop an operational manual that identifies specific hazards that will or may be encountered, and that specifies practices and procedures designed to minimize or eliminate risks. Personnel should be advised of special hazards and should be required to read and follow the required practices and procedures.

Laboratory personnel safety practices and techniques must be supplemented by appropriate facility design and engineering features, safety equipment, and management practices as determined by risk assessment.

BIOSAFETY PRACTICES & PROCEDURES

Members of the IBC will inspect the research facility (visual inspection) to ensure that the space meets regulatory standards. Inspections of BSL-1 laboratories, facilities, and farms are valid for 5 years while inspections of BSL-2 and BSL-3 laboratories, facilities, and farms which are not used for select agent work are valid for 3 years.

The inspectors will ask the PI who is responsible for the space a series of questions (verbal inspection) to verify that the proper biosafety procedures are being adhered to by all laboratory personnel. The visual and verbal inspection checklists for each type of containment facility can be found on the Biosafety Office [webpage](#). Noncompliance with the items in the checklists will result in suspension or termination of IBC approval. The checklist items are self-explanatory; however, the following sections provide further details for some of the items.

Biohazard Warning Signs and Postings

Anyone entering areas where biohazardous materials are used must be aware of the potential hazards. All BSL-2 and BSL-3 laboratories/facilities will display similar signage. The sign or door placard will include the biohazard symbol, the biosafety containment level, the PI's contact information and any required procedures for entering and/or exiting the laboratory.

Sharps Handling

Written policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. The greatest risks when using sharps are

accidental injection and the creation of aerosols. Laboratory supervisors should adopt engineering and work practice controls that reduce the risk of sharps injuries.

- Needles and syringes may only be used when there is no reasonable alternative. Safety needles and syringes must be used in these instances.
- Sharps must be kept away from fingers as much as possible. Sharps must never be bent, sheared, or recapped. Needles should never be removed from syringes after use. If a contaminated needle must be recapped or removed from a syringe, a mechanical device, such as forceps, must be used.
- Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
- Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
- Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps.
- Do not overfill sharps containers beyond their designated capacity.
- Filled sharps containers must be autoclaved or decontaminated by an approved method before disposal.

Use of Biological Safety Cabinets

All procedures that have the potential to create infectious aerosols must be performed inside a BSC or other containment device unless a project has been approved by the IBC with the room serving as primary containment. All BSCs must be certified annually.

BSCs are designed to protect personnel, research products, and the environment. The BSC accomplishes this protection by directional airflow and HEPA filters. HEPA stands for high efficiency particulate air and can filter 0.3 micron particles at 99.97% efficiency. HEPA filters are even more efficient at trapping both smaller and larger sized particles. The HEPA filter removes airborne particles from the air but does not remove chemical fumes. Some chemicals may even compromise the HEPA filters. The *BMBL* provides more detailed information on the different types of BSCs.

There are other types of hoods that share some similarities with a BSC, but cannot provide all three categories of protection. For example, a chemical fume hood is designed to protect personnel by removing chemical vapors and aerosols from the work area. However, it does not

protect the environment from biohazardous materials because it does not typically have a HEPA filter. Laminar flow hoods should also not be used when working with biohazardous materials because these types of hoods can only protect the research product, as they do not protect the researcher or others in the vicinity.

If your cabinet is hard-ducted to an in-house exhaust system, then you will want to keep your BSC running at all times and you will need to leave the sash up to ensure proper exhaust of your laboratory. Contact your building manager or the Biosafety Office if you are unsure about whether you need to leave your BSC on or if you need to turn it off. If your BSC can be turned off, always make sure it is disinfected prior to shutting it down. For the most consistent contamination control and safe operation, BSCs should be operating 24 hours a day, 7 days a week.

Preparing to Work in a BSC:

- Turn on the BSC and wait for the air to purge (10 minutes) to prevent product contamination.
- Put on a lab coat or solid front gown and gloves (additional PPE such as eye or respiratory protection may be required based upon risk assessment).
- Ensure that the BSC has been certified within the last 365 days.
- Ensure that the drain valve is closed so that any spills are contained within the cabinet.
- Adjust the seat to the proper height (armpits should be level with the view screen; feet should be on the floor or foot rest).
- Disinfect all surfaces within the BSC (work surface, walls, interior of sash).
 - Note: Bleach can be corrosive. Rinse with sterile water if bleach is used.
- Set up work items so that clean items are on one side of the cabinet and dirty items are on the other, leaving the middle section as a work area.
- Ensure that nothing is blocking the front grill of the BSC.
- Ensure that the view screen is in the proper position as determined by the cabinet's manufacturer.

Working in a BSC:

- Limit traffic in the area when the BSC is in use.

- Conduct work in the center of the cabinet as far back as comfortably possible.
- Move slowly and deliberately while working.
- Move arms straight in, perpendicular to the opening and wait for one minute to start manipulations; do not use sideways or sweeping motions in order to prevent airflow disruption.
- Use good aseptic microbial technique.
- Do not use open flames or flammable gas in the BSC. Open flames inside of a BSC can disrupt the airflow, compromising protection of both the worker and the material being handled. Open flames are extremely dangerous around flammable materials, such as ethanol, which is often found in a BSC. Electric incinerators, touch plate microburners, and disposable sterile instruments are excellent alternatives.
- Only use grounded electrical equipment inside of the cabinet.
- If a spill occurs, follow the laboratory spill cleanup procedures.
- If the BSC alarm sounds while the cabinet is in use, close or cover all vessels containing biohazardous material and report the problem to the PI or lab manager.

Disinfection and Cleanup of the BSC:

- Place all disposable items that have been exposed to biohazardous material in a biohazard bag within the cabinet.
- Disinfect items before bringing them outside of the BSC, including the outside of the biohazardous waste bag and PPE.
- Be sure to allow adequate disinfection time for the disinfectant used. Alternatives for bleach or alcohol are preferred. Bleach has a tendency to corrode stainless steel and even 70% alcohol evaporates very quickly.
- Disinfect all surfaces in the cabinet, including the work surface, interior walls, and inside of sash with a disinfectant and proper contact time that is appropriate for the particular biohazardous material used. **Never put your head inside the BSC!**
- UV lights should not be the only means used to disinfect a BSC because the light cannot penetrate organic material and its efficiency decreases over time. Be sure the UV light is turned off before beginning work and when occupants are in the laboratory because exposure to UV light for a prolonged period will cause burns.

- Schedule routine thorough cleanings of the BSC to include cleaning the pan underneath the work surface.
- If the BSC does not remain on at all times, leave the blower on for an additional 5-10 minutes once disinfection is complete.

Laundry

The OSU IBC recognizes that all University employees have the right and the need to know the properties and potential safety issues and health problems associated with the substances to which they may be exposed. Therefore, non-disposable materials (e.g., lab coats) must be autoclaved or treated with an appropriate disinfectant for the appropriate contact time prior to being taken to any laundry facility. Lab coats are not to be taken home for washing.

Housekeeping

Due to the risks posed to non-laboratory personnel (e.g., vendors performing equipment maintenance, Facilities Management personnel, custodians, etc.), these individuals must not enter BSL-2 or BSL-3 laboratories/facilities after normal business hours or without a laboratory manager or PI present. The IBC recognizes the need for routine maintenance and service on equipment and general upkeep of laboratories and facilities (e.g., light bulb replacement, floor waxing, equipment maintenance, etc.). In these cases, the PI and/or laboratory manager is responsible for continual supervision of all non-laboratory personnel while in BSL-2 and BSL-3 spaces. Non-laboratory personnel may not have unescorted access to these research spaces.

Biohazard Spill Cleanup

Each laboratory in which biohazardous materials are used must have appropriate equipment and supplies on hand for managing spills and incidents involving biohazardous materials. Permanent equipment should include a safety shower (if appropriate), eyewash, and a hand-washing sink and supplies. A Biohazard Spill Kit should also be kept on hand and immediately accessible. The supplies available in a Biohazard Spill Kit should include, but are not limited to:

- a copy of the biohazard spill clean-up protocol;
- disposable shoe covers (booties);
- absorbent material, such as absorbent paper towels, granular absorbent material, etc. (a disposable or cleanable scoop will be needed for granular absorbent material);

- all-purpose disinfectant, such as normal household bleach (freshly diluted 1:10) or other appropriate disinfectant;
- something disposable or easily disinfected such as tongs, forceps, manila folders, etc. for picking up broken glass, other contaminated sharps, or contaminated absorbent material;
- autoclavable biohazard waste bags; and
- biohazard spill warning signs.

Note: All non-disposable items must be autoclavable or compatible with the disinfectant to be used.

An IBC-approved fill-in-the-blank spill cleanup protocol is available on the Biosafety Office webpage at: <https://research.okstate.edu/research-compliance/ibc/documents/spill-sop.pdf>.

Personal Exposure to Infectious Material

In the event that a substance enters the mouth, eyes, lungs, or penetrates/comes in contact with the skin, follow the instructions below and seek immediate medical attention.

- Alert others in the laboratory.
- Remove all contaminated PPE and clothing.
- Treat the exposed area by washing with soap and water or flushing with water.
- Post a warning sign on the laboratory door.
- Report the incident to the PI.
- Seek medical attention.

On-site emergency assistance can be obtained by dialing 911. Bring the appropriate Safety Data Sheet (SDS) to the provider to aid in medical treatment.

Decontamination

All employees who through their work generate biohazardous waste must strictly adhere to the OSU waste disposal guidelines delineated below.

All biohazardous waste must be decontaminated before disposal. Common decontamination methods include heat sterilization (e.g., autoclaving), chemical disinfection, incineration, or tissue digestion. When using an autoclave for steam sterilization, the waste should be treated for a minimum of 15 minutes at 121°C at 15 psi. The sterilization time will be dependent upon the volume of the waste and the concentration of organisms.

Animal Carcasses, Tissues, and Bedding: All animal carcasses and tissues that have been exposed to biohazardous materials must be decontaminated before disposal. Animal bedding and other wastes may also require decontamination before disposal as dictated by risk assessment. All waste should be autoclaved, incinerated, or tissue digested as applicable.

Liquids: Decontaminate all liquid biohazardous materials by autoclaving or treating with the appropriate chemical disinfectant if autoclaving is not an option. Following decontamination, the liquids may be disposed of by pouring them down the drain to the sanitary sewer with cold running water.

Disposable Solid Items: Collect all non-sharp disposable items (e.g., gloves, Kimwipes, etc.) that have been contaminated with biohazardous materials in leak-proof, autoclavable biohazard bags and decontaminate by autoclaving. After autoclaving, two sides of the biohazard bag must be labeled with yellow stickers (see image below), which can be obtained from the departmental safety officer or EHS. After affixing the labels, the waste is to be double-bagged in dark trash bags and placed in a solid waste container.

FORM: OAC 252:515-23-4		
The material contained in this bag meets the definition of "Treated Bio-medical waste." The material has undergone steam sterilization or chemical disinfection to render the waste harmless and biologically inert. I further certify by my signature that this container <u>DOES NOT</u> contain liquids, scalpels, sharps, glass, blades, or needles which might puncture this bag/container.		
Dept.	Signed:	Date:

Non-disposable or Reusable Items: Decontaminate non-disposable or reusable items (e.g., equipment, glassware, benchtops, etc.) contaminated with biohazardous materials by using a chemical disinfectant (e.g., 10% bleach, a quaternary ammonium compound, alcohol, etc.). Choose a chemical disinfection that is appropriate for the specific biohazardous material being used and make sure to use the appropriate contact time for each disinfectant.

Sharps and Broken Glass: Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Collect all sharps in an approved, rigid, leak-proof container that is autoclavable if contaminated with biohazardous material. After decontamination, the sharps container will be picked up upon request by EHS. For broken glassware that is not contaminated with biohazardous material, collect in a rigid, leak-proof container labeled "broken glass." Package it in a cardboard box that has been sealed and labeled "broken glass," and discard in the regular trash.

Transportation of Biohazardous Materials

When transporting biohazardous materials outside of the laboratory, materials must be placed in a primary non-breakable, leak-proof, sealed container and enclosed in a non-breakable secondary container. The secondary container should be lined with absorbent materials if liquids are being transported.

Surplus Laboratory Equipment

Surplus laboratory equipment is often sold to the public. Thus, it is important to take precautions to ensure that potentially infectious materials are not transferred to unsuspecting individuals. Before surplus items are picked up, the PI or laboratory manager must:

- ensure that the equipment is completely empty;
- properly decontaminate all surfaces using an effective disinfectant; and
- remove all biohazard warning stickers from the outside of the equipment.

Laboratory Decommissioning

When vacating a laboratory space, it is the PIs responsibility to ensure that the space has been properly cleaned and is ready for the next occupant. The following steps should be carried out before vacating the space.

- Transfer or dispose of all biohazardous agents/recombinant material and decontaminate the laboratory (i.e., floors, benchtops, drawers, etc.) using an effective disinfectant (e.g., 10% bleach, 70% ethanol, etc.).
- Decontaminate all solid biohazard trash by autoclaving and dispose of in accordance with OSU policy.
- Decontaminate all liquid biohazard waste by autoclaving or chemical treatment.
- Decontaminate all remaining equipment using an effective disinfectant.
- Remove biohazard door signage and equipment labels.

Please contact the Biosafety Office should you have any questions regarding the lab decommissioning process.

EMERGENCY & INCIDENT RESPONSE

IMPORTANT CONTACT INFORMATION

Emergency (Police, Ambulance, Fire) – 911 or (405) 744-6523

Biosafety Personnel:

Biosafety Officer – (405) 744-3736

Assistant Biosafety Officer – (405) 744-3203

University Health Services (UHS) – 744-7665

Stillwater Medical Center (SMC) – (405) 372-1480

EHS Occupational Health & Safety – (405) 744-3017

LABORATORY EMERGENCY RESPONSE

Laboratory emergencies require the response of emergency personnel in addition to other responders and include fires, explosions (with or without an accompanying fire), gas leaks, and medical emergencies.

Dialing 911

When emergencies occur, it is critical that laboratory personnel reaction quickly to any situation by securing work areas, closing all doors, reporting the emergency immediately to 911, and providing situation information for emergency responders. Calls to 911 from campus telephones are answered by the OSUPD dispatcher. However, calls to 911 from off-campus telephone and cell phones are answered by the Stillwater Police Department, which forwards these calls to the OSUPD dispatcher. If you call 911 from an off-campus telephone or cell phone, communicate that you are calling about an emergency on the OSU campus. OSUPD can be reached directly at (405) 744-6523 from an off-campus phone.

Emergency Severity

To assist emergency responders, laboratory personnel must provide responders with an indication of how serious the event is. Basically, the responders need to know what occurred and how severe the event is.

Every emergency reported by laboratory personnel should include a description of the event, the laboratory level, and one of the following laboratory situation codes:

Code Green: The emergency involves no risk for emergency responders due to laboratory chemicals or hazardous agents. The laboratory situation is normal.

Code Yellow: The emergency involves a situation inside a laboratory area that involves a health or safety risk to emergency responders. However, the emergency is contained inside the laboratory area and does not present a hazard outside of the laboratory containment area. Containment measures are operating normally.

Code Red: The emergency is a health or safety risk to emergency responders and everyone in the building because the material is not contained by the building systems. Uncontrolled fires are a Code Red emergency in any event.

The following are examples of how emergencies should be reported.

Reporting party: “This is Jane Doe at B52 Boomer Hall. We have a person here with chest pains and we need an ambulance. This is a BSL-2 lab. This is a Code Green emergency.”

Reporting party: “This is John Doe at 747 Hanger Hall. We have had a small flask of an infectious agent shatter and cut one of our laboratory researchers. We have the bleeding stopped, but there researcher is still in the lab because of the spilled material. The agent is contained in the BSL-3 lab. Everyone else is out of the area. This is a Code Yellow emergency.”

Reporting party: “This is Orville Wright at 1903 Flyer Hall. We have a fire in a biological safety cabinet. Everyone has evacuated the room, but the fire is out of control. This is a Code Red emergency.”

R.A.C.E. Model

When confronted with fire or other laboratory emergencies, laboratory personnel should follow the R.A.C.E. model.

1. **Rescue** those in immediate danger, without becoming a victim.
2. **Alarm** - activate the nearest pull station or call 911.
3. **Contain** the fire or emergency by closing doors.
4. **Extinguish** the fire if you are trained to do so and it is a small fire. Otherwise, evacuate the area.

Evacuation Routes & Procedures

Emergencies that may require evacuation include but are not limited to bomb threats, chemical spills, civil disturbances, earthquakes, explosions, fires, gas leaks/eruptions, severe weather (e.g., tornadoes, high winds, lightning strikes, etc.) and other natural disasters, terror-related events, and workplace violence.

Upon notification of an emergency that requires evacuation, laboratory personnel must, if possible:

1. Immediately cease laboratory procedures and secure the work area.
2. Decontaminate and remove all containers of infectious materials from biosafety cabinets and place them into autoclaves, incubators, refrigerators, or freezers as quickly as possible. Biosafety cabinets should remain on if they were operating at the time of the emergency.
3. Turn off all gas burners.
4. Laboratory containment ventilation systems should be left on.
5. Leave the building as quickly as possible and assemble as a group in a safe area outside of the building and stay together. **DO NOT REENTER THE BUILDING FOR ANY REASON.** Anyone missing should be noted and reported to the fire department incident commander immediately.

Laboratory personnel evacuated from the building in an emergency who may be contaminated with a chemical, infectious agent, or radioactive material due to an exposure or release are to:

1. prevent others from becoming exposed or contaminated;
2. take self-protective measures by removing contaminated clothing, if possible, and place in garbage bags for autoclaving; and
3. wait for emergency decontamination by emergency responders.

Note: Under no circumstances should emergency response personnel be exposed or contaminated without their knowledge.

Emergency responders will not enter the building until there is some reliable information regarding the situation inside the building and the risk to personnel. PIs or laboratory personnel having specific information on the situation inside the building (e.g., what occurred, what material is involved, where the situation is, etc.) or missing people should report the information to the incident commander at the command post. Any fire or police officer will be able to provide the location of the command post.

LABORATORY INCIDENT RESPONSE

Laboratory incidents can typically be handled by laboratory personnel and can include biological spills, non-serious injuries, and personal exposures to infectious agents.

Seeking Medical Attention

For critical injuries, call 911. For all other injuries, seek medical attention as indicated below.

Medical Treatment during work hours (i.e., M-F, 8:00 a.m.-5:00 p.m.):

- OSU employees/students, go to University Health Services at 1202 West Farm Road
- Non-OSU employees/students, go to Stillwater Medical Center at 1323 West 6th Avenue

Medical Treatment outside of work hours (i.e., evenings, weekends, & holidays):

- All individuals go to Stillwater Medical Center at 1323 West 6th Avenue

Reporting Procedures

All incidents must be reported immediately to the laboratory PI or manager. The IBC requires all laboratory biosafety incidents occurring in a BSL-2 or BSL-3 space to be reported to the BSO within 48 hours. Large spills of biohazardous material (i.e., >10 mL in volume) that occur outside of primary containment should be immediately reported to the BSO. A Laboratory [Incident Report Form](#) must then be completed and submitted to the Office of University Research Compliance within one week. An [Employee Injury Report](#) may be submitted in lieu of the Laboratory Incident Report form if an employee is injured.

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