Montana State University-Bozeman

Biosafety Manual
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Certification and Approvals

Signature of IBC Chair

Mike Babcock

Date

Signature of Biosafety Officer

Kirk Lubick

Date

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<th>Previous Review Dates</th>
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<tr>
<td>May 1, 2014</td>
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May 12, 2016
Chapter 1 - Biological Safety Program: Purpose, Scope, and Responsibilities

Purpose

The purpose of this Biosafety Manual is to define policies and procedures pertaining to the use of biological materials in research at Montana State University (MSU). These policies and procedures are designed to safeguard personnel and the environment from biologically hazardous materials without limiting research.

The work practices, procedures, and policies specified in this manual are based on regulatory requirements and accepted biosafety practices. Implementation of these measures will reduce the likelihood that an incident involving a biological agent will occur and will fulfill regulatory biosafety requirements. Laboratory work usually involves potential exposure to biological hazards, as well as to chemical and radiological hazards. Consequently, this manual should be used in conjunction with the MSU Chemical Safety Manual and Radiation Safety Manual, respectively.

For information about specific biological safety programs for operations not covered in this manual, contact the Biosafety Officer (BSO).

Scope

This manual applies to all MSU research activities involving biological agents. All faculty, staff, students, and visitors who work on MSU sponsored projects or at MSU facilities are included in the scope of this manual.

Biological agents include all infectious biological agents (bacteria, fungi, parasites, prions, rickettsia, viruses, etc.) that can cause disease in humans or pose significant environmental or agricultural impact, as well as the toxins derived from biological agents. Additionally, recombinant DNA; human or non-human primate tissues, fluids, cells, or cell cultures; transgenic plants or animals; and any work with animals and their tissues, which are known to be reservoirs of zoonotic diseases, are covered by the procedures and policies in this manual.

Biological Safety Program Goals

The Biosafety Program is designed to provide guidance on the safe handling and containment of all activities involving biohazardous materials, to minimize the risks of laboratory acquired infections, and to maintain compliance with all regulations pertaining to recombinant DNA and biohazardous materials. The mission of the Biosafety Program at MSU is to ensure a safe environment for individuals working with
biohazardous materials and to ensure the protection of the community and environment by preventing exposure to biohazardous materials. To accomplish this, the Biosafety Program provides technical advice to Principal Investigators on laboratory containment, security, and safety procedures. Other aspects of the Biosafety Program include developing emergency response plans for handling spills and personnel containment, overseeing laboratory inspections to ensure safe laboratory standards are maintained, and providing Biosafety training, recombinant DNA regulations training, and Bloodborne Pathogen training.

**Roles and Responsibilities**

Success of the Biosafety Program requires a team effort involving the IBC, Principal Investigators, laboratory workers, the Occupational Health Program (OHP), and Safety and Risk Management (SRM). Principal Investigators are responsible for the health and safety of personnel who work under their supervision and occupy their laboratory space. MSU administration and the IBC endorse this manual and encourage active participation in maintaining high standards at MSU.

**Director of Research Compliance (IO)**

The IO has overall responsibility for:

- Oversight for the control of biohazardous materials in the research laboratories and for ensuring that a comprehensive biosafety program is in place for the safe handling of all biohazardous materials.
- Direct functional responsibility for the IBC and Biosafety Program.
- Develops and ensures communication between the IBC and other research related committees.
- In consultation with IBC chair appoints committee members to the IBC.

**Institutional Biosafety Committee**

The IBC is responsible for reviewing and approving practices and protocols for the handling of rDNA and potentially biohazardous materials at all research facilities at MSU. The IBC carries out these functions pursuant to requirements set forth by the National Institutes of Health (NIH), the Centers for Disease Control and Prevention (CDC), and Occupational Safety and Health Administration (OSHA).

The IBC’s responsibilities include:
• Overall oversight of the Institutional Biosafety Program at MSU, including development of new, and review of existing, policies and procedures designed to enhance the Biosafety Program.
• Reviews and approves training programs.
• Coordinates the biological safety requirements with other campus-wide committees (e.g., IACUC) or programs (e.g., Occupational Health Program).
• Reviews and approves new research protocols involving rDNA and biohazardous material in accordance with guidelines established by the OSHA, USDA, CDC, NIH, and MSU, as well as maintains protocol approval and reviews modifications.
• Sets required containment levels for research projects. Generally, biosafety levels (BSL) established by the CDC and NIH will be used as the level of containment; however, the IBC has the authority to increase or decrease the level of containment according to the project’s specific circumstances.
• Investigates violations of biosafety procedures or policies and significant accidents or illnesses involving biological agents.
• If appropriate, recommends disciplinary action to the proper MSU officials.
  o NIH, concerning rDNA exposures incidents
  o NIH’s Office of Biotechnology Activities, an IBC update

Biosafety Officer

The BSO is responsible for developing, leading, directing, and managing a comprehensive biosafety program for MSU. The biosafety program must meet NIH, CDC, USDA, OSHA, any other granting agency, Federal, State, and local requirements. The program includes close cooperation and interaction with committees approving research protocols and procedures for use of human subjects (Institutional Review Board (IRB)), Institutional Animal Care and Use Committee (IACUC), and Radiation Safety Committee (RSC). The BSO will provide guidance and consultation to assess the risk of working with potentially biohazardous materials. The BSO interacts with the research, teaching, and diagnostic community to inform and ensure compliance with State and Federal reporting or audit requirements, and to inspect and correct deficiencies when noted.

The BSO duties include:

• The inspection of the physical facilities and containment equipment for compliance with general CDC guidelines for Biosafety Level (BSL) and Animal Biosafety Level (ABSL) laboratories for research and diagnostic work using developed laboratory inspection checklists.
• Review of laboratory biosafety manuals and standard operating procedures (SOPs) for compliance with guidelines for BSL and ABSL procedures.
• Provides general guidance about health and safety standards, and provides the biosafety review for all research proposals presented to the IBC.
Per SPPM S80.12, S80.13, S80.14, helps ensure that biohazards, sharps, and glass wastes are properly treated, transported, and disposed of outside of laboratory facilities and after leaving the laboratory buildings per applicable state and federal regulations.

Maintains list of approved biosafety laboratories with inspection dates and results.

Responsible for assisting the PI to develop appropriate Lab-specific biosafety manuals for all activities using potentially biohazardous materials.

The BSO regularly reports on the Biosafety Program to the IBC. The BSO’s report should include routine operational updates and any significant problems or violations of the regulatory mandates or IBC requirements on any research-related accidents or illnesses that have occurred.

Principal Investigators

Principal Investigators (PIs) are responsible for the health and safety of all personnel and compliance with all applicable regulations and the criteria established in this manual in their laboratories.

The PI:

- Notifies the IBC and obtains prior IBC approval for work involving recombinant DNA and/or biohazardous material and conforms to all terms and conditions of IBC approval. Ensures that all laboratory personnel are adequately trained in the practices and techniques required to ensure safety and the procedures for dealing with accidents.
- Informs the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested.
- Ensures that individuals working in the facility are experienced and proficient in handling biological agents.
- Makes available to all laboratory personnel the protocols that describes the hazards and the precautions to be taken.
- Ensures that the required safety practices, techniques, engineering controls and PPE are provided and employed.
- Ensures that laboratory hazards are effectively communicated to laboratory personnel and controls are in place to minimize risks associated with these hazards.
- Notifies the Biosafety Officer of any spills or incidents involving biological agents that result in exposure to laboratory personnel or the public, or release to the environment.
- Ensures that biological materials are disposed of according to regulations, as outlined in this manual.
- Ensures that biohazardous materials to be transported are packaged and shipped in accordance with regulations.
Occupational Health Program

The Occupational Health Program (OHP) is primarily responsible for establishing and performing appropriate medical surveillance for all personnel performing research or supporting research such as animal care workers, facilities, Police and Public Safety. Surveillance is required at the time of hire or transfer into the research environment and periodically depending on the work environment, occupational exposure and risk for each position or job category. OHP is responsible for reporting all biological exposure incidents to the appropriate personnel.

In addition to performing medical surveillance, OHP is also responsible for:

- Coordinates with Montana Occupational Health to provide medical evaluations and surveillance program for personnel working in the facility.
- Files Workman’s Compensation reports.
  Provides annual N95 Respirator training and certification

Laboratory Workers

Laboratory workers are the most important element in developing and maintaining a safe laboratory environment. Laboratory workers are responsible for their own health and safety, as well as that of their coworkers. An incident caused by one laboratory worker can have a widespread effect on others.

Laboratory workers are expected to:

- Participates in and completes all required training to ensure that they are adequately trained.
- Fully understands the biological agents and procedures used in the laboratory and the risks associated with exposure.
- Follows all laboratory practices, protocols and complies with all applicable policies, procedures, and guidelines.
- Informs the JRL Manager of any potential problems with the operating procedures or equipment which may result in the creation of a potential hazard.
- Completes any necessary medical surveillance.
- Reports thefts, security incidents, accidents, spills, or contamination incidents to JRL Manager, PI and RO/ BSO.
Chapter 2 - Approval of Research Projects

Who Needs Approval

The IBC reviews and approves many areas of biologically related activities which may include research, teaching, and diagnostic activities.

The IBC defines biohazardous materials to include all infectious organisms (e.g., bacteria, chlamydia, fungi, parasites, prions, rickettsia, and viruses) that can cause disease in humans, animals, or plants, or have significant negative environmental or agricultural impact. Work with materials that may harbor infectious organisms, such as human or primate tissues, fluids, cells, or cell cultures are also considered biohazardous material.

Potentially biohazardous materials include, but not limited to, all of the categories below. Projects involving materials included in any of these categories must be submitted for IBC approval prior to initiating the project.

- Recombinant DNA (rDNA)
- Genetically modified organisms. Including, but not limited to:
  - Animals, plants, invertebrates, or other organisms created/used by MSU employees.
  - Transgenic field trials involving any genetically modified organisms that are introduced into the environment, including planting of deregulated items in the field.
  - Field testing of plants engineered to produce pharmaceutical and industrial compounds.
- Pathogens/infectious agents (e.g., human, animal, plant, and other).
- CDC or USDA designated Select/Biological Agents and Toxins. Please note that possession, use, or transfer of Select Agents and Toxins entails additional requirements – contact the ORC for additional information.
- Human and non-human primate cells including cell lines, tissue, blood and potentially infectious fluids.
- Work with animals or vectors known or suspected to be reservoirs of RG2 or RG3 infectious agents when such work increases potential exposure risks to personnel or other animals.

When working with potentially infectious agents and human subjects or experimental animals, IBC review is necessary in addition to review by the Institutional Animal Care and Use Committee (IACUC) or the Institutional Review Board (IRB).
Principles which Govern the IBC

The IBC operates upon the following regulations/guidelines:

- NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH guidelines)
- Biosafety in Microbiological and Biomedical Laboratories (BMBL).
- 42 CFR Part 73, Possession, Use, and Transfer of Select Agents and Toxins.
- 29 CFR 1910 Bloodborne Pathogen Standard

The planning and implementation of safety protocols to prevent laboratory-acquired infections and to eliminate the spread of contamination must be part of every laboratory’s routine activities and biosafety manual. No work should be considered so important that it jeopardizes the well-being of the worker or the environment.

The handling of biological agents and recombinant DNA requires the use of precautionary measures dependent on the agents involved and the procedures performed. It is the purpose of this manual to provide background information and guidelines to be used in conjunction with other resources for the evaluation, containment, and control of potentially biohazardous materials in laboratories.

**IBC protocol**

A PI applying for IBC approval for research, teaching, or diagnostic activities needs to submit a completed IBC protocol. In order for the application to be processed, it must be signed (electronic) by the PI and any supplemental materials must be included.

A PI applying for approval of teaching activities involving potentially biohazardous material must contact the BSO. The BSO will assist the PI in developing appropriate biosafety training for students. The PI is responsible for ensuring that all students are all trained prior to working with the agents. The BSO will act as a resource to assist the PI in developing a Biosafety Manual and performing a facility review.
Requests for modifications in activities after approval

All modifications to currently approved research and diagnostics activities are required to have IBC review and approval prior to implementation. Minor changes that do not increase the risk to workers, the community, and/or the environment may be processed as an administrative approval performed by the IBC Chair and/or BSO.

Examples of significant modifications may include; the addition of potentially biohazardous materials, and the addition of materials or procedures that may increase the risks of the research. Administrative modifications may be approved by the IBC Chair or the BSO. Examples of administrative modifications may include the addition of personnel, and change of laboratory room (if change is to an equivalent and approved facility).

The IBC modification approval is valid until the end of the original approval period (3 years).

Reports of unexpected adverse events

All unanticipated/adverse events should be reported to the BSO and IBC chair in writing as well as any actions taken on the part of the researcher as a response to the adverse event. NIH Guidelines require that the PI report any significant events to IBC representatives and OBA within 30 days.

Notification

Three prior to the expiration of an approved protocol, the PI will receive an e-mail notification that their approved protocol is about to expire. PIs desiring to continue their research are responsible for completing a new IBC protocol and returning it to the IBC coordinator in time for review before the expiration date. A second notice will be sent to the PI two months prior to the expiration date.

One month prior to the expiration a final notification will be emailed. If the PI fails to submit a new protocol and gains IBC approval prior to the expiration data, all work on the project must be discontinued.

Renewals

A renewal notice serves as a mechanism for the PI to provide an annual update of the research occurring on an IBC protocol and this form is sent to the PI listed on the original approval the first and second year after initial approval of a protocol. The PI is asked to list any proposed deviations from the protocol as initially approved (or since the last renewal notice); changes in laboratory staff working on the project; if there has been any problems/adverse events; and to provide a summary of the project over the last year.
If there are significant deviations from the protocol, especially deviations that affect the containment level (i.e., new study organisms, a new host-vector-donor system, or any other modifications that may affect the containment level), the PI will need to submit a modification or in some cases may need to submit a new IBC protocol to cover the additional experiments.

When a project is renewed as part of the annual update process, all new lab staff must complete lab safety training.
Chapter 3 - List of Regulations and Guidelines

The following is a summary of federal, state, and local agency regulations and guidelines that either regulate or provide guidelines covering the use of biological agents:

- Centers for Disease Controls and Prevention and the National Institutes of Health: Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition, 2009. This document contains guidelines for microbiological practices, safety equipment, and facilities that constitute the four established biosafety levels. The BMBL is considered the standard for biosafety and is the basis for this manual.

- National Institutes of Health: Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines). This document provides guidelines for constructing and handling recombinant DNA molecules (rDNA) and organisms containing rDNA. Although these guidelines are not subject to regulatory enforcement, institutions that receive any NIH funding for rDNA research are required to comply with these guidelines as a condition of funding. The NIH Guidelines requires that each institution establish an Institutional Biosafety Committee with the authority to approve proposed rDNA research using the NIH guidelines as the minimum standard.

- Occupational Safety and Health Administration: Bloodborne Pathogens. This regulation covers occupational exposure to human blood and other potentially infectious materials, including human tissue and cells. OSHA specifies a combination of engineering controls, work practices, and training to reduce the risk of infection. Personnel potentially exposed to human blood and other potentially infectious material must be offered immunization against hepatitis B and receive annual training. Personnel who work with HIV or hepatitis B in a research laboratory must receive additional training and demonstrate proficiency in working with human pathogens.

Select Agent Rule

Department of Health and Human Services: 42 CFR Parts 42 and 43 Possession, Use, and Transfer of Select Agents and Toxin; Final Rule; and the Department of Agriculture’s Animal and Plant Health Inspection Service: 7 CFR Parts 331 and 9 CFR Parts 121, Agricultural Bioterrorism Protection Act of 2002: Possession, Use, and Transfer of Biological Agents and Toxin; Final Rule. These regulations require institutions that possess, use, or transfer certain biological agents and toxins (“select agents”) to be registered and approved by DHHS and/or APHIS. Specific requirements are described in Chapter 10.
Other Regulatory Requirements

**U.S. Department of Transportation and the International Air Transportation Authority:** These organizations have strict requirements governing the shipment and transportation of hazardous materials, including biological agents. Chapter 11 provides information on shipping regulations.

**Centers for Disease Control and Prevention:** The CDC has established specific regulatory requirements for importation or transportation of etiologic agents, which include a permit application that must be submitted and approved prior to any such importations. The federal regulation governing the importation of etiologic agents is USPHS 42 CFR - Part 71 Foreign Quarantine. Part 71.54, Etiologic agents, hosts, and vectors.

**U.S. Department of Agriculture, Animal and Plant Health Inspection Service, and Veterinary Services:** USDA, APHIS, and VS regulate the importation of animals and animal-derived materials to ensure that exotic animal and poultry diseases are not introduced into the United States. Generally, a USDA veterinary permit is needed for materials derived from animals or exposed to animal-source materials. Materials that require a permit include animal tissues, blood, cells or cell lines of livestock or poultry origin, RNA/DNA extracts, hormones, enzymes, monoclonal antibodies for in vivo use in non-human species, certain polyclonal antibodies, antisera, bulk shipments of test kit reagents, and microorganisms, including bacteria, viruses, protozoa, and fungi. Exceptions to this requirement are human and non-human primate tissues, serum, and blood.

**U.S. Department of Commerce:** The DOC has specific regulatory requirements for exportation of biological materials. These regulations are both agent and country specific and must be followed strictly.

**Institutional Biosafety Committee:** The IBC has publicized a number of specific policies and procedures that are incorporated into this document as requirements or have been included as appendices.
Chapter 4 - Risk Group Classifications

The Biosafety in Microbiological and Biomedical Laboratories (BMBL) defines the three primary hazardous characteristics associated with a biological agent as the following:

- The capability of an agent to infect and cause disease in a susceptible human or animal host;
- The virulence of an agent as measured by the severity of disease; and
- The availability of preventive measures and effective treatments for the disease.

By taking the route of transmission of the disease into consideration, a standardized methodology was developed to classify biological agents into four different risk groups. Knowing the risk group of an agent assists researchers and safety professionals in determining the appropriate safety protocols to be followed.

Risk Group 1
Agents not associated with disease in healthy adult humans.

Risk Group 2
Agents associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available.

Risk Group 3
Agents associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk).

Categories

Biohazards are biological agents or biologically derived infectious materials that present a risk or potential risk to the health of humans, plants or animals. Biological agents have the ability to replicate and give rise to potentially large populations in nature when small numbers are released from a controlled situation.

The following is a listing of the potentially hazardous biological agents and materials. PIs should follow the instructions in the IBC protocol form application carefully to ensure that all appropriate sections of the
application are completed. If a PI intends to use biological agents that are not listed in this section, he or she should contact the IBC or BSO for advice regarding proper completion of the “Biological Use Authorization”.

- Human, animal, and plant pathogens
- Viruses, including oncogenic and defective viruses
- Rickettsia
- Chlamydia
- Bacteria, including those with drug-resistant plasmids
- Fungi
- Parasites
- Undefined or other infectious agents, such as prions
- All human blood, blood products, tissues, and certain body fluids
- Cultured cells (all human or certain animal, including non-human primates) and the potentially infectious agents these cells may contain
- Allergens
- Toxins (bacterial, fungal, plant, etc.)
- Clinical and diagnostic specimens
- Infected animals and animal tissues
- Non-human primates and any tissues derived from them (can transmit Herpes B virus)
- Sheep and any tissues derived from them (can transmit Coxiella burnetii, the causative agent of Q-fever)

Recombinant DNA (rDNA) Materials

Generation or Use of rDNA

The NIH’s Guidelines for Research Involving Recombinant DNA Molecules is the regulatory reference for recombinant DNA (rDNA) research in the United States.

Use of Animals

The use of animals in research requires compliance with the “Animal Welfare Act,” administered by the USDA’s Animal and Plant Health Inspection Service (APHIS); the “Public Health Service Policy on Humane Care and Use of Laboratory Animals,” administered by NIH’s Office of Laboratory Animal Welfare (OLAW); and all applicable state or local regulations covering the care and use of animals. All protocols involving the use of live animals must be reviewed and approved by the IACUC before their implementation.

Transgenic Animals
PIs who create transgenic animals, as well as PIs who use transgenic animals at ABSL-2, or at ABSL-1 if not considered exempt under the NIH Guidelines (Section III-E, Appendix C-VIII) must complete an IBC application and submit it to the IBC for approval prior to initiation of the experiment. In addition, the IACUC must approve the protocol.

**Tissue Culture/Cell Lines**

**Risk Group 1/Biosafety Level 1 (BSL-1)**

The following are considered Risk Group 1 cell lines and can be handled using BSL-1 containment:

- Non-primate cell lines origin and do not harbor a primate virus or are nor contaminated with bacteria, mycoplasma, or fungi

**Note:** All human and non-human primate cell lines require BSL-2 containment as they potentially may harbor previously undefined pathogens.

**Risk Group 2/Biosafety Level 2 (BSL-2)**

When cell cultures are known to contain an etiologic agent or an oncogenic virus, the cell line can be classified at the same level as that recommended for the agent or virus. The CDC has recommended that all cell lines of human origin be handled at BSL-2.

The following are identified as Risk Group 2 and must be handled at BSL-2:

- Primate cell lines derived from lymphoid or tumor tissue
- All cell lines exposed to or transformed by a primate oncogenic virus
- All clinical material (e.g., samples of human tissues and fluids obtained after surgical resection or autopsy for use in organ culture or establishment of primary cell cultures)
- All primate tissue
- All cell lines new to the laboratory (until proven to be free of all biohazardous agents)
- All virus and mycoplasma-containing primate cell lines

**Risk Group 3/Biosafety Level 3 (BSL-3)**

When cell cultures are known to contain any Risk Group Three biological agent, the cell line will be classified at the same level as that recommended for the biohazardous agent.
The following are examples of biological materials identified as Risk Group 3 and must be handled at BSL-3:

- High titer HIV work
- St. Louis encephalitis virus
- Venezuelan equine encephalomyelitis virus
- M. tuberculosis
- Concentrated Lentivirus or Lentiviral vectors with high likelihood of aerosol formation.
- Francisella tularensis

Human Tissue and Cell Culture

Working with Human Tissues and Cells

All unfixed human tissue and cells are to be assumed to be infectious (the concept of “universal precautions”) and must be handled using BSL-2 practices and procedures. Persons who are exposed to these materials in the laboratory are considered to have potential exposure to bloodborne pathogens, such as human immunodeficiency virus (HIV) and hepatitis B virus (HBV), and must be included in the Bloodborne Pathogens program. These persons must be offered the hepatitis B vaccination and receive annual bloodborne pathogens training.

Cell Culture

Human or animal pathogens may be associated with cell or organ cultures. Cell cultures known (or suspected) to contain an etiologic agent or an oncogenic virus are classified at the same biosafety level as that recommended for the agent.

The following cell cultures and tissues require BSL-2 or higher containment and procedures:

- All cultured cells derived from human sources, including immortalized and “well established” cell lines.
- All cultured cells derived from non-human primates, primate lymphoid, or tumor tissue.
- All cultured cells exposed to or transformed by a primate oncogenic virus.
- All clinical materials, such as samples of human tissue obtained from surgery, biopsy, or autopsy.
- All primate and sheep tissue.
- All uncharacterized cultured cells new to the laboratory until proven to be free of infectious agents.
- All virus-containing primate cultured cells.
- All mycoplasma-containing cultured cells.
Chapter 5 – Routes of Transmission

Routes of Transmission

The risk of exposure to biological agents in a research environment depends on a number of parameters (e.g., pathogenicity, virulence, infectious dose, communicability, subject’s susceptibility, route of transmission, etc.). In general, the biosafety procedures used are designed to prevent such exposures by containing the agents. To properly design the containment, it is important to recognize the potential routes of transmission for the given agent.

Skin and Mucous Membrane Contact

Decanting of liquids, pipetting, removal of screw caps, vortex mixing, streaking agar plates, and inoculation of animals, may result in the generation of infectious droplets, as well as direct contact with infectious material. Eye contact is also considered a route of exposure.

Ingestion

Splashing of material into the mouth and indirect oral exposure through touching the mouth with contaminated hands can result in the ingestion of infectious material. Storage of food or drinks in laboratories with biological agents, as well as storage of utensils and eating and drinking in the lab, can also result in ingestion of infectious material. Mouth pipetting also presents a high risk for ingestion of infectious materials.

Percutaneous Inoculation

Use of syringes and needles are considered the greatest risk of exposure through inoculation. Accidental inoculation can also occur as a result of cuts and scratches from contaminated items including syringes used for animal inoculations, as well as animal bites.

Inhalation

Many procedures have the potential for generation of aerosols, including sonication, centrifugation, “blowing out” of pipettes, heating inoculating loops, and changing litter from the cages of infected animals.
Chapter 6 - Biosafety Principles

Biosafety Principles are used to provide safe methods for managing infectious agents in the laboratory environment where they are being handled or maintained. The purpose of Biosafety Principles is to reduce or eliminate exposure of laboratory workers, other people, and the outside environment to potentially hazardous agents. The four elements of containment are engineering controls, standard operating procedures, personal protective equipment, and administrative controls.

Engineering Controls

Engineering controls includes facility design, Biological Safety Cabinets (BSCs), enclosed containers, safety centrifuge cups, and other engineering controls designed to minimize exposure to biological agents.

Primary Engineering Controls

The protection of personnel and the immediate laboratory environment from exposure to infectious agents. Primary engineering controls are the most effective at minimizing exposure when workers are trained on the proper use of such equipment and the equipment is regularly inspected and maintained. Biological Safety Cabinets (BSC) are the most important primary engineering control for protection of personnel and the laboratory environment, and most also provide product protection.

Secondary Engineering Controls
Protecting the laboratory’s external environment from exposure to infectious materials is accomplished by a combination of biosafety principles and the facility design plays a major role. Facility air flow, access control, sinks for hand washing, lab benches are impervious to water resistant to heat, and self-closing doors are some examples of secondary engineering controls.

**Personal Protective Equipment (PPE)**

Personal protective equipment (PPE) includes safety eyewear, face shields, gloves, appropriate respiratory protection, and lab coats. This equipment is used to supplement the containment provided by laboratory practices and safety equipment.

PPE is designed to protect laboratory workers from serious exposure to biohazardous materials and should be used in conjunction with appropriate engineering and administrative controls. At a minimum, staff must use lab coats, safety glasses, and gloves whenever there is a potential for skin contact, splash, or aerosols.

**Standard Operating Procedures**

A manual of written standard operating procedures (SOPs) for the laboratory, in combination with this manual and the CDC/NIH publications Biosafety in Microbiological and Biomedical Laboratories and Guidelines for Research Involving Recombinant DNA Molecules, provide general requirements for working with biological agents. However, because these cover relatively general topics, individual laboratories are required to develop laboratory-specific SOPs that cover the biosafety concerns and laboratory procedures for that particular laboratory.

For example, laboratory-specific SOPs should address safe manipulation of specific organisms, specific exposure control methods, and specific decontamination and waste-handling requirements. The laboratory- specific SOPs do not need to duplicate the more general SOPs contained in this manual or the CDC/NIH documents, but should serve as supplements.

**Administrative Controls**

Administrative controls are policies and procedures designed to assist with the safe handling of potentially hazardous biological materials. They include training, medical surveillance, vaccinations, access control, etc.
Chapter 7 - Biosafety Level Containment Laboratories

Four Biosafety Levels (BSLs) represent combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the operations performed and the documented or suspected routes of transmission of the infectious agents, as well as for the laboratory function or activity. The recommended biosafety level for an organism represents the conditions under which the agent can be ordinarily handled safely.

NIH’s Guidelines for Research Involving Recombinant DNA Molecules classifies “human etiologic agents” on the basis of their relative pathogenicity. Agents are categorized into four risk groups (RG)

As a general rule, a biosafety level should be used that matches the highest RG classification of the organisms involved. For example, work with vaccinia virus, a Risk Group 2 (RG2) agent, should be conducted at BSL-2 or higher; simultaneous work with E. coli (RG1), Epstein-Barr virus (RG2), and Mycobacterium tuberculosis (RG3) should be conducted at BSL-3.

Descriptions of biosafety levels, as well as assigned biosafety levels for specific organisms, are contained in the CDC/NIH document, Biosafety in Microbiological and Biomedical Laboratories (BMBL). The BMBL outlines four biosafety levels, summarized below:

<table>
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<tr>
<th>Biosafety Level</th>
<th>Agents</th>
<th>Practices</th>
<th>Safety Equip.</th>
<th>Facilities</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Not known to cause disease in healthy adults; RG1</td>
<td>Standard microbiological practices</td>
<td>Use basic personal protective equipment including laboratory coats, disposable gloves and as necessary eye protection</td>
<td>Open bench top, sink required</td>
</tr>
<tr>
<td>2</td>
<td>Associated with human disease, which is rarely serious and for which preventive or therapeutic interventions are often available; RG2</td>
<td>BSL-1 practice plus: Limited access, Biohazard warning signs, Sharps precautions, Biosafety manual</td>
<td>Primary barriers: Class I or II BSCs or other containment used for manipulations of agents that cause splashes or aerosols of infectious materials. PPE: lab coats; gloves; eye/face protection as needed.</td>
<td>BSL-1 plus: Autoclave available</td>
</tr>
<tr>
<td>Level</td>
<td>Description</td>
<td>BSL-2 Practice plus</td>
<td>BSL-3 Practice plus</td>
<td>BSL-3 plus</td>
</tr>
<tr>
<td>-------</td>
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<td>---------------------</td>
<td>---------------------</td>
<td>------------</td>
</tr>
<tr>
<td>3</td>
<td>Associated with human disease for which preventive or therapeutic interventions may be available; RG3</td>
<td>Primary barriers: Class I or II BSCs or other physical containment devices used for all manipulations of agents. PPE: protective lab clothing; gloves; respiratory protection as needed.</td>
<td>Primary barriers: All procedures conducted in Class III BSCs or Class I or Class II BSCs in combination with full-body, air-supplied, positive-pressure personnel suit.</td>
<td>Other requirements outlined in BMBL</td>
</tr>
<tr>
<td></td>
<td>Baseline serum</td>
<td>Controlled access</td>
<td>Clothing change before entering Shower on exit All material decontaminated on exit from facility</td>
<td>- Separate building or isolated zone - Dedicated supply/exhaust, vacuum, and decontamination systems - Other requirements outlined in BMBL</td>
</tr>
<tr>
<td>4</td>
<td>Agents are likely to cause serious or lethal human diseases for which preventive or therapeutic interventions are not usually available; RG4</td>
<td>Baseline serum</td>
<td>Baseline serum</td>
<td>Baseline serum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controlled access</td>
<td>Decontamination of all waste</td>
<td>Physical separation from access corridors - Self-closing, double-door access - Exhausted air not recirculated - Negative airflow into laboratory</td>
</tr>
</tbody>
</table>

**Note:** Consult the BMBL for a more complete description of the four biosafety levels, as well as recommended biosafety levels for specific organisms.
The foundations of protective practices in a laboratory lie in an individual’s laboratory experience, technical knowledge, personal work habits, and attitude toward laboratory safety. Unlike administrative controls, which are behaviors dictated by regulation or laboratory policy, the term “protective behavior” is used to define an innate part of each individual worker’s personal approach to the laboratory environment. As such, “protective behaviors” form the first and most important line of defense against injury or exposure in the laboratory.

**Basic Laboratory Practices**

Prudent practices and good techniques are of primary importance in laboratory safety. Both are based on sound technical knowledge, experience, common sense, and an attitude of courtesy and consideration for others.


At a minimum, the seven basic rules of biosafety, based on the National Research Council’s Prudent Practices document, should be the basis of any personal laboratory work ethic.

1. Do not mouth pipette.
2. Manipulate infectious fluids carefully to avoid spills and the production of aerosols.
3. Restrict use of needles, syringes, and other sharps to those procedures for which there are no alternatives; dispose of sharps in leak- and puncture-proof containers.
4. Use lab coats, gloves, safety eyewear, and other personal protective equipment.
5. Wash hands after all laboratory activities, following the removal of gloves, and immediately following contact with infectious agents.
6. Decontaminate work surfaces before and after use, and immediately after spills.
7. Do not eat, drink, store foods, or smoke in the laboratory.

**Laboratory Practice and Technique**

The most important element of containment is strict adherence to standard microbiological practices and techniques.
Persons working with infectious agents or infected materials must be aware of potential hazards and be trained and proficient in the practices and techniques required for handling such material safely. The PI is responsible for ensuring that laboratory personnel are properly trained; the PI may delegate the provision of training to the laboratory supervisor, but the responsibility remains with the PI.

Each laboratory should develop an operational manual identifying specific hazards that will or may be encountered and specifying practices and procedures designed to minimize or eliminate risks. Personnel should be advised of special hazards and should be required to read and to follow the required practices and procedures. A scientist trained and knowledgeable in appropriate laboratory techniques, safety procedures, and hazards associated with the handling of infectious agents must direct laboratory activities.

When standard laboratory practices are not sufficient to control the hazard associated with a particular agent or laboratory procedure, additional measures may be needed. The PI is responsible for selecting additional safety practices, which must be in keeping with the hazard associated with the agent or procedure.

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

**Note:** Although each individual is responsible for his or her own safety, the PI has ultimate responsibility for ensuring that persons working in the laboratory are adequately trained and that they follow the prescribed safety measures.

**Laboratory Housekeeping and Personal Hygiene**

With laboratory space at a premium, dedicated bench space is a rarity. For those who do not have to share work space, personal safety is greatly enhanced by keeping the area neat, clean, and orderly. Injuries and exposures are more likely to occur in poorly maintained, disorderly areas.

If work space is shared, the importance of maintaining a neat, clean area increases significantly. Coworkers must rely on one another to maximize efficiency and safety. Personal materials should be properly labeled, waste discarded, and the shared space disinfected or cleaned prior to leaving it for the next user.

The following guidelines should be observed in the laboratory:

- Routine housekeeping ensures work areas are free of significant sources of contamination and hazards.
- Housekeeping procedures should be based on the highest degree of risk to which personnel and experimental integrity may be subjected.
- Laboratory personnel are responsible for cleaning laboratory benches, equipment, and areas that require specialized technical knowledge.
- Access to exits, sinks, eyewashes, emergency showers, and fire extinguishers must not be blocked.
- The workplace should be free of physical hazards.
- Electrical safety is a priority, especially as it relates to the use of extension cords. Equipment should be properly grounded. Overloaded electrical circuits and the creation of electrical hazards in wet areas are to be avoided.
- Surfaces should be clean and free of infrequently used chemicals, glassware, and equipment.
- Unnecessary items on floors, under benches, or in corners should be removed.
- All compressed gas cylinders should be properly secured.

Personal hygiene, including proper handwashing techniques, is also a means by which to enhance personal protection in the laboratory. Scrubbing immediately after degloving ensures that contamination of the hand by glove micropuncture or prior exposure is neutralized before being spread.

The laboratory is also an inappropriate place to perform personal cosmetic tasks, such as applying makeup, cleaning or trimming fingernails, or brushing hair. These activities provide new opportunities for exposure and contribute to retrograde contamination of the laboratory environment.

### Universal Precautions

Prudent practices often overlap with a set of practices known as “universal precautions.” The overarching universal precaution defined by the Bloodborne Pathogens (BBP) Standard should be adopted by all laboratory personnel.

Universal precautions require that all human blood and tissues be handled as though they are infectious. Adopting and applying universal precautions to all laboratory reagents clearly creates a heightened awareness of potential risk and adds another level of caution to activities involving reagents.

### Biological Hazard Information

Laboratory workers must be knowledgeable of the hazards associated with the biological agents present in the laboratory and have hazard information available to them. The following are sources of hazard information for biological agents.
Storage and Labeling of Biological Agents

Biological agents must be stored using leak proof and sealed container. Containers must be clearly labeled with the identity of the agent and should include the universal biohazard symbol (see below) as physical space on the container permits. At a minimum, secondary (or outside) containers must include the universal biohazard symbol (identity of contents is also desirable).

Freezers, refrigerators, and other storage areas must also be labeled with the biohazard symbol; exceptions to this policy will be considered on an individual basis by the IBC. Waste and contaminated equipment or other objects to be decontaminated must also be labeled with the biohazard symbol.

Universal Biohazard Symbol

The OSHA Bloodborne Pathogen Standard specifically requires that containers of human blood or other potentially infectious material (OPIM), contaminated waste, and refrigerators, freezers, and other storage containers used to store or transport blood or OPIM be labeled with the universal biohazard symbol (fluorescent orange or orange-red).

Biohazard Labels and Signs

Each laboratory must have a sign at the entrance that provides safety information to visitors and service personnel. Room signs must contain designations for all laboratory hazards in use within the laboratory (carcinogens, acutely toxic agents, reproductive hazards, biohazards, radioactive materials, lasers, and magnetic fields). Safety and Risk will prepare the signs for each door in accordance with the requirements of NFPA 704.

Biohazard signs will be posted at the following:

- Entrances to laboratories and animal rooms that use agents classified as BSL-2 or BSL-3.
- Cages or animal rooms used for housing animals infected with BSL-2 or BSL-3 agents. For a sample of MSU door signage, see Appendix T.

Certain other areas and pieces of equipment within a laboratory may also require signs. Refrigerators, freezers, cabinets, and other storage facilities require the biohazard symbol whenever they are used to
store infectious agents of Risk Group 2 or higher; human blood or blood products; unfixed tissues; cell or organ cultures; body fluids; or excreta. Large pieces of equipment for handling such materials (e.g., centrifuges, biological safety cabinets) must be similarly labeled.

**Microbial Agents**

- The CDC/NIH’s Biosafety in Microbiological and Biomedical Laboratories (BMBL) has descriptions of biosafety levels and recommended biosafety practices for specific biological agents.

**Toxins**

Isolated biological toxins are chemical hazards, although many such toxins produce adverse effects at doses significantly below that of “traditional” laboratory chemicals. MSDSs for a specific toxin should be obtained from the vendor upon receipt of the toxin. Some biological toxins require IBC approval before work is initiated. For further details please go to Appendix F for more details.

- Toxicology textbooks, such as Casarett & Doull’s Toxicology, are also good sources of hazard information for toxins.

**Security and Inventory of Biological Agents**

In recent years, a number of highly publicized incidents involving biological materials have increased both public concerns and regulatory oversight concerning the security of biological agents. Even though many of the agents used in research laboratories do not pose a real risk to health and safety of the workers or the public, the perception of such risks is of great importance.

At MSU, each PI must develop site-specific criteria that safeguard all biological materials, regardless of their risk group, from unauthorized removal. It is the PI’s responsibility to ensure that his or her laboratory implements sufficient security measures and procedures to prevent unauthorized access to biological agents.

*Select agents and other higher-risk biological agents and toxins must be stored in a locked container, and a detailed inventory must be maintained per CDC requirements.* In many instances, during the
application review process, the IBC will review the proposed acceptable safeguards and either approve or recommend enhancements to the proposed plans.

**Prevention of Aerosols and Droplets**

Handling of liquids or dry powders generally is likely to generate aerosols or droplets. In practice, high-energy procedures, such as centrifuging, vortexing and mixing, tend to produce aerosols that stay airborne for extended periods and are small enough to be inhaled, while low-energy procedures, including opening containers and streaking plates, produce droplets that settle quickly on surfaces, skin, and mucous membranes.

**Utilization of Biological Safety Cabinets**

In general, the following guidelines are recommended when using biological safety cabinets (BSCs):

- The BSC should be certified when it is installed or after it is moved, and annually thereafter (for information on cabinet certification contact the Biosafety Officer).
- The magnahelic gauge should be checked regularly. This gauge will normally run at a relatively fixed value. When it deviates significantly, the cabinet should not be used until the cause of the deviation has been identified and fixed.
- Personnel should understand how the BSC works.
- Personnel should be familiar with the safe and effective use of any UV lamps inside the BSC and use appropriate precautions to avoid UV-related injuries.
- The BSC’s protective airflow pattern should not be disrupted. Rapid arm movement, nearby workers, and open laboratory doors may disrupt the airflow pattern and reduce the cabinet’s effectiveness.
- Work and the necessary materials should be planned to minimize the need to exit and reenter the work area.
- Accumulation of materials in the BSC work area should be minimized to reduce turbulence and ensure proper laminar air flow.
- The BSC should be left running whenever the cabinet is in use.
- Work surface should be wiped with 70% alcohol before use. Each item needed for the planned procedures should be wiped off and placed in the BSC.
- After the work volume is set up, the BSC should run for at least 5 minutes to allow for stabilization of air flow before any procedures are begun.
- If a piece of equipment, such as a centrifuge or blender, will create air turbulence in the BSC, it should be placed in the back one-third of the cabinet. All other work should be stopped while this equipment is operating.
- Open flames are not allowed in the work area because they create air flow turbulence that may compromise sterility. In addition, the heat buildup may damage the HEPA filters. Electric devices, such as loop sterilizers, are often satisfactory alternatives to open flames.
- A pan with disinfectant and/or a sharps container should be placed inside the BSC for pipette/sharps disposal. Vertical pipette discard canisters on the floor outside the cabinet should be avoided.
- Contaminated and clean items should be segregated, and personnel should work from “clean to dirty.” The biohazardous waste collection bag should be in a rigid container. Do not block air flow into the front and rear exhaust grilles.
- All spills in the cabinet should be cleaned immediately. Work should not resume for 10 minutes.
- When work is complete, all materials should be removed from the BSC and all interior surfaces should be wiped with 70% alcohol, or other appropriate disinfectants.
- Gloves must be removed before exiting the BSC, after touching or handling contaminated materials.
- Laboratory coat must be removed and hands thoroughly washed before leaving laboratory.

**Utilization of Pipettes**

Pipettes are used for volumetric measurements and the transfer of fluids that may contain infectious, toxic, corrosive, or radioactive agents. Laboratory-associated infections have occurred from oral aspiration of infectious materials, mouth transfer via a contaminated finger, touching face (eyes, nose, etc) and inhalation of aerosols. Exposure to aerosols may occur when liquid from a pipette is dropped onto the work surface; when cultures are mixed by pipetting; or when the last drop of an inoculum is blown out.

The following outlines safe pipetting techniques to minimize the potential for exposure to hazardous materials:

- Never mouth pipette. Always use a pipetting aid.
- If working with biohazardous or toxic fluid, confine pipetting operations to a biological safety cabinet.
- Always use cotton-plugged pipettes when pipetting biohazardous or toxic materials, even when safety pipetting aids are used.
- Do not prepare biohazardous materials by bubbling expiratory air through a liquid with a pipette.
- Do not forcibly expel biohazardous material out of a pipette.
- Never mix biohazardous or toxic material by suction and expulsion through a pipette.
- When pipetting, avoid accidental release of infectious droplets.
- Use “to deliver” pipettes rather than “to contain” pipettes, which require “blowout.” Be careful not to dislodge the residual liquid.
• Do not discharge material from a pipette at a height. Whenever possible, allow the discharge to run down the container wall
• Place contaminated, reusable pipettes horizontally in a pan containing enough liquid disinfectant to completely cover them. Autoclave the pan and pipettes as a unit before processing them for reuse.
• Discard contaminated, broken, or intact Pasteur pipettes and broken glass in a sharps container.
• Dispose of the container properly when it is, at most, three-fourths full.
• Pans or sharps containers for contaminated pipettes should be placed inside the BSC, if possible.
• Proper procedures for disposal of plastic pipettes are presented in Chapter 9.

Utilization of Centrifugation

Hazards associated with centrifuging include mechanical failure and the creation of aerosols. To minimize the risk of mechanical failure, centrifuges must be maintained and used according to the manufacturer’s instructions. Users should be properly trained and operating instructions that include safety precautions should be prominently posted on or near the unit.

Aerosols are created by activities such as filling centrifuge tubes, removing plugs or caps from tubes after centrifugation, removing supernatant, and re-suspending pellets. A significant aerosol hazard can be created if a tube breaks during centrifugation.

To minimize the generation of aerosols when centrifuging biohazardous material, the following procedures are recommended:

• Use sealed tubes and safety buckets that seal with O-rings. Before use, inspect tubes, O-rings, and buckets for cracks, chips, erosions, bits of broken glass, etc. Do not use aluminum foil to cap centrifuge tubes because it may detach or rupture during centrifugation.
• Fill and open centrifuge tubes, rotors, and accessories in a biological safety cabinet. Avoid overfilling centrifuge tubes to prevent closures from becoming wet. After tubes are filled and sealed, wipe them down with disinfectant.
• In the event of breakage during centrifugation, the unit should be decontaminated prior to reuse.
• Always balance buckets, tubes, and rotors properly before centrifugation.
• Avoid decanting or pouring off supernatant; unless the supernatant must be retained, use a vacuum aspirator with appropriate in-line reservoirs and filters.
• Work in a biological safety cabinet when re-suspending material. Use a swirling rotary motion rather than shaking. If shaking is necessary, wait a few minutes to permit the aerosol to settle before opening the tube.
• Small, low-speed centrifuges may be placed in a biological safety cabinet during use to reduce the aerosol escape. High-speed centrifuges pose additional hazards. Precautions should be taken to filter the exhaust air from vacuum lines, to avoid metal fatigue resulting in disintegration of rotors,
and to use proper cleaning techniques and centrifuge components. Manufacturers’ recommendations must be meticulously followed to avoid metal fatigue, distortion, and corrosion.

- Avoid the use of celluloid (cellulose nitrate) tubes with biohazardous materials. Celluloid centrifuge tubes are highly flammable and prone to shrinkage with age. They distort on boiling and can be highly explosive in an autoclave. If celluloid tubes must be used, an appropriate chemical disinfectant must be used.

Utilization of Cryostats

Use of cryostats is very common in many research laboratories. These devices may pose potential hazards associated with sharp cutting edges and cold environments and should be handled with extra care.

The following guidelines should be followed when using cryostats:

- Frozen sections of unfixed human tissue or animal tissue infected with an etiologic agent pose a risk because freezing tissue does not necessarily inactivate infectious agents. Use of freezing propellants under pressure is not recommended with frozen sections because they may cause spattering of droplets of potentially infectious material.
- Appropriate gloves should be worn during preparation of frozen sections.
- When working with human or infected animal tissue, consider the contents of the cryostat to be contaminated and decontaminate it frequently with 70% alcohol.
- Consider trimmings and sections of tissue that accumulate in the cryostat to be potentially infectious and remove them during decontamination.
- Defrost and decontaminate the cryostat with a tuberculocidal hospital disinfectant once a week and immediately after use with tissue known to contain bloodborne pathogens, M. tuberculosis, or other infectious agents.
- Handle microtome knives with extreme care. Stainless steel mesh gloves should be worn when changing knife blades.
- Solutions used for staining potentially infected frozen sections should be considered contaminated.

Utilization of Inoculating Loops

Flaming inoculating loops can result in spatter and the release of aerosols and droplets. Use of an electric microincinerator is the preferred alternative, to minimizing this issue.
Use of Absorbent Materials

Work surfaces should be covered with absorbent paper or “diaper” sheets to collect splashes and drips and to minimize the spread of contamination. The absorbent paper should be changed at the end of the laboratory procedure as part of the final cleanup, or at least daily during use.

Utilization of Miscellaneous Aerosol-Producing Devices and Activities

Use of any of the devices listed below results in considerable aerosol production. Blending, cell-disrupting, and grinding equipment should be used in a BSC when working with biohazardous materials.

Blenders

Safety blenders, although expensive, are designed to prevent leakage from the bottom of the blender jar. They provide a cooling jacket to avoid biological inactivation and can withstand sterilization by autoclaving.

- If blender rotors are not leak-proof, they should be tested with sterile saline or dye solution prior to use with biohazardous material.
- The use of glass blender jars is not recommended because of the potential for breakage. If they must be used, glass jars should be covered with a polypropylene jar to prevent spraying of glass and contents in the event the blender jar breaks. The blender must be operated within a secondary containment basin.
- A towel moistened with disinfectant should be placed over the top of the blender during use.
- When opening blenders, be cognizant of potential contamination hazards in the form of droplets that might become airborne or fall on the surfaces; liquid residue on the cap; and possible expansion of the volume due to aeration.
- Before opening the blender jar, allow the unit to rest for at least one minute to allow the aerosol to settle.
- Placing the blender in a BSC will provide protection against airborne hazards and placement of a tray lined with absorbent pads would assist with contamination control.
- The device should be decontaminated promptly after use.

Lyophilizers

Depending on lyophilizer design, aerosol production may occur when material is loaded into or removed from the lyophilizer unit.

- If possible, sample material should be loaded in a BSC.
• The vacuum pump exhaust should be filtered to remove any hazardous agents or, alternatively, the pump can be vented into a BSC.
• After lyophilization is complete, all surfaces of the unit that have been exposed to the agent should be disinfected.
• If the lyophilizer is equipped with a removable chamber, it should be closed off and moved to a BSC for unloading and decontamination.
• Handling of cultures should be minimized and vapor traps should be used wherever possible.

**Sonicators**

Sonication is the use of sound-wave energy for dispersion, disruption, or inactivation of biological materials, such as viruses. Sonicators generate sound waves at very high frequencies (~20,000 + Hz range), which is outside normal hearing range. The following are hazards associated with sonicators:

• Noise: Although the 20,000-Hz frequency is outside normal hearing range, there are other sources of noise, such as vibration from any loose equipment or other items on the bench or the liquid itself. If the noise levels are high, normal hearing protection devices should be worn.
• Aerosols: Aerosols present a more serious potential hazard and must be taken into consideration. Precautions listed for blenders and lyophilizers should be observed.

**Ampoules**

Opening ampoules containing liquid or lyophilized culture material should be performed in a BSC to control any aerosol produced. Sealed-glass ampoules used to store biohazardous material in liquid nitrogen have exploded, causing eye injuries. The use of polypropylene tubes (cryovials) eliminates this hazard.

These tubes are available dust-free or pre-sterilized and are fitted with polyethylene caps with silicone washers. Heat-sealable polypropylene tubes are also available.

• Gloves must be worn when opening ampoules or cryovials.
• To open a sealed-glass ampoule, nick the neck of the ampoule with a file, wrap it in disinfectant-soaked disposable towel, hold the ampoule upright, and snap it open at the nick.
• Reconstitute the contents of the ampoule by adding liquid slowly to avoid aerosolization of the dried material.
• Mix the contents without bubbling and withdraw it into a fresh container. Discard the disposable towel and the ampoule’s top and bottom as medical waste.
Loop Sterilizers and Bunsen Burners

Sterilization of inoculating loops or needles in an open flame generates small-particle aerosols that may contain viable microorganisms.

- Alternatively, disposable plastic loops and needles may be used for culture work where electric incinerators or gas flames are not available.
- Continuous flame gas burners should not be used in a BSC. These burners can produce turbulence that disturbs the cabinet’s protective airflow patterns. Additionally, the heat produced by the continuous flame may damage the HEPA filter. If a gas burner must be used, one with a pilot light should be selected. Electric sterilizers should also be considered.
Chapter 9 – Engineering Controls

Primary Engineering Controls

Biological Safety Cabinets (BSC)

BSCs are important in providing containment and safety protections in a laboratory, and BSC are considered one of the most critical pieces of safety equipment in Biosafety Level (BSL) Containment laboratories. BSCs differ from chemical and laminar flow hoods (clean hoods) in that they always offer personnel protection. BSCs are designed to contain aerosols generated during work with biological material through the use of laminar air flow and high efficiency particulate air (HEPA) filtration. Proper use of BSCs provides a high level of protection for laboratory personnel from exposure to biological material while providing some protection from contamination of the material being handled within the work environment.

Three types of BSCs (Class I, II, and III) that offer different levels of protection are available for use in BSL containment laboratories. Open-fronted Class I and Class II BSCs are partial containment devices that provide a primary barrier offering significant levels of protection to laboratory personnel and to the environment when used in combination with good laboratory technique. The gas-tight Class III BSC, or glove box, provides the highest level of protection to personnel, the environment, and the product.

The Class I BSC is suitable for work where there is a need for protection from the biological material, but not for protection of the product. It provides protection to personnel and the environment from contaminants within the BSC but does not protect the work within the cabinet from “dirty” room air.

The Class II BSC protects the material being manipulated inside the cabinet (e.g., cell cultures, microbiological stocks) from external contamination. It meets requirements to protect personnel, the environment, and the product. The two basic types of Class II BSCs are Type A and Type B. The major differences between the two types may be found in the percent of air that is exhausted or recirculated and the manner in which exhaust air is removed from the work area.

The gas-tight Class III BSC, or glove box, provides the highest level of protection to personnel, the environment, and the product. It is the only unit that provides a total physical barrier between the product and personnel. It is used with high-risk biological agents and when absolute containment of highly infectious or hazardous material is required.

It is important to note that laminar flow hoods (clean hoods) or chemical fume hoods must not be utilized for work with biohazardous agents. Laminar flow hoods provide product protection by ensuring the product is exposed only to HEPA-filtered air. They do not provide protection to personnel or the laboratory environment. Chemical fume hoods only provide personal protection by directional airflow into the fume
hoods and preventing chemical fumes from exiting out of the hood. They do not provide HEPA filtered air and therefore do not provide suitable protection to personnel, the product, or the environment.

**BSC Owners Responsibilities and BSC Maintenance**

Proper operation and maintenance of a BSC requires knowledge of how the system operates, as well as training and experience in effective techniques for working within the BSC without compromising its functions. Additional details concerning the design and use of BSCs are provided in Appendix C.

Two specialized forms of quality control are strongly recommended for all BSCs:

- At least daily, or each time the cabinet is operated, the operator or user should observe the magnahelic gauge and note its relative position. Magnahelic gauges measure the pressure drop across the outlet HEPA filter and are important indicators of filter integrity and loading. The gauge will typically indicate the same measurement over a long period of time. A significant change in the reading over a short period of time may indicate clogging or a leaking filter. In such cases, the hood should not be used until the problem is identified and resolved. If the BSC located within a laboratory does not have a magnahelic gauge, users must understand the operation of the airflow monitor, controls, and alarm settings.

- Annually, the cabinet must be certified by an ASEPSIS. The certification process is quick and relatively inexpensive and ensures that the BSC is meeting its operating specifications and providing maximum protection. In addition, ASEPSIS provides service and preventive maintenance for BSCs and can often forecast expensive requirements like HEPA filter replacements, allowing PIs to budget for the event.

- The recertification must be completed before the current certification expires. If the certification lapses, the BSC may not be used for BSL-2 or higher procedures until it is recertified. The lab will report the lapsed recertification to the Biosafety Officer immediately. The Biosafety Officer will inform the PI and lab workers not to use the BSC and to post a label “DO NOT USE” and assist the lab to get the BSC recertified through ASEPSIS. Unless a good reason exists for more frequent certification, a one-year certificate life is appropriate. The certificate will generally expire on the last day of the month in which the certification was performed, one year later (for example, a certificate issued on June 2, 2015 will expire on June 30, 2016).

Principal Investigators are responsible for ensuring the proper maintenance of BSCs. BSCs used as primary barriers must be certified annually by ASEPSIS. Contact the Biosafety Officer for information about ASEPSIS or other BSC-related information.
Secondary Engineering Controls

Facility Design

The design of a facility is important in providing a barrier to protect people working inside and outside the laboratory, as well as to protect people or animals in the community from infectious agents that may be accidentally released from the laboratory. Facility design must be commensurate with the laboratory's function and the recommended biosafety level for the agent being used or stored.

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 BSL-1 and BSL-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 decontamination equipment (e.g., autoclave*); and sinks for handwashing. In BSL-3 facilities, additional safeguards, such as directional airflow, airlock-controlled entry and exiting, a shower for personnel to shower out may be required.

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 ensure directional airflow; air treatment systems to decontaminate or remove agents from exhaust air; controlled access zones; an airlock at the laboratory entrance; or separate buildings or modules for physical isolation of the laboratory building itself.

*Note: It is Biosafety policy that autoclaves used to sterilize biohazardous materials be validated monthly using a sporulation test and that validation records be kept. Biohazardous materials can also be disposed of in a red bag as medical waste without autoclaving which will be picked up by SRM.
Chapter 10 - Personal Protective Equipment (PPE)

Personal protective equipment (PPE) must be provided without cost to personnel. Although not a substitute for the use of BSCs and good laboratory practices, PPE is considered a primary barrier to infectious agents and proper use will reduce the likelihood of infection. PPE is the least-desirable exposure control method because its failure results in direct exposure to the agent.

PPE is most effective when used to supplement primary control methods such as biological safety cabinets, safety centrifuge cups, and other containment devices. Appropriate clothing may also protect the experiment from contamination.

The following are considered PPE:

**Face Protection**

Goggles or safety glasses with solid-side shields in combination with masks, or chin-length face shields or other splatter guards, are required for anticipated splashes, sprays, or splatters of infectious or other hazardous materials to the face. Wearing contact lenses is inappropriate in the laboratory setting.

**Laboratory Clothing**

Laboratory coats, smocks, scrub suits, and gowns are considered laboratory clothing.

- Long-sleeved garments should be used to minimize the contamination of skin or street clothes and to reduce shedding of microorganisms from the arms.
- In circumstances where it is anticipated that splashes may occur, the garment must be resistant to liquid penetration to protect clothing from contamination.
- If the garment is not disposable, it must be capable of withstanding sterilization, in the event it becomes contaminated.
- Additional criteria for selecting clothing include comfort, appearance, closure types and location, antistatic properties, and durability.
- Protective clothing must be removed and left in the laboratory before leaving for non-laboratory areas.
- Disposable clothing should be available for visitors, maintenance, and service workers in the event it is required. All protective clothing should be discarded in the laboratory, disinfected, or laundered by the facility.
- Personnel must not launder laboratory clothing at home.
Gloves

Gloves must be selected on the basis of the hazards involved and the activities to be conducted.

- Gloves must be worn when working with biohazardous and/or toxic materials and physically hazardous agents.
- Temperature-resistant gloves must be worn when handling hot materials, dry ice, or materials being removed from cryogenic storage devices.
- Delicate work requiring a high degree of precision dictates the use of thin-walled gloves.
- When working with hazardous materials, the glove should overlap the lower sleeve and cuff of the laboratory garment. A long-sleeved glove or disposable arm-shield may be worn for further protection of the garment.
- In some instances, double gloving may be appropriate. If a spill occurs, hands will be protected after the contaminated outer gloves are removed.
- Gloves must be disposed of when contaminated, removed when work with infectious materials is completed, and never worn outside the laboratory.
- Disposable gloves must not be washed or reused.
- Protection from contact with toxic or corrosive chemicals may also be required. For assistance in glove selection, call Safety and Risk.

Respirators

Respirators are selected based on the hazard involved and the protection factor required. Certain laboratory and clinical situations require respiratory protection to prevent inhalation of infectious agents.

Regulations, as well as good safety practice, require that personnel be medically evaluated, specifically trained, and fit tested prior to wearing respiratory protective equipment.

Contact Safety and Risk if respiratory protective equipment is required or if there are questions about the respiratory protection program.

Footwear and Miscellaneous Clothing Guidelines

Open-toed shoes or sandals are not allowed in the lab. In addition, wearing shorts or other clothing that exposes the lower legs is generally considered unsuitable in laboratories because it increases the potential for skin contamination and absorption of contaminants.
Chapter 11 - Laboratory Training

Training is a critical component of any integrated biological safety program. Training is intended to provide the understanding, technical knowledge, and tools that the trainee can use to improve his or her daily laboratory safety practices.

At a minimum, all personnel working with biological materials at MSU must have training in the following areas prior to the start of their experiments:

- Knowledge of this biosafety manual
- Experimental procedures to be used
- Decontamination and spill clean-up procedures
- Safe handling methods for any infectious agent and/or recombinant DNA (rDNA) they might be handling
- Proper methods for transporting infectious agents and other biohazardous materials
- Bloodborne Pathogens Standard (if they work with human blood or blood products, unfixed tissue, body fluids, organ, or primary tissue and/or samples contaminated with bloodborne pathogens)
- Other specialized training as Biosafety deemed appropriate by the IBC or the BSO.

The PI is responsible for ensuring that his or her employees receive proper training in the biohazards and controls specific to his or her laboratory and the safe conduct of the experimental procedures to be used. The Biosafety Program provides different types of training associated with the various laboratory programs at MSU.

Mandated General Biosafety Training

This training is required by law and/or policy and must be obtained through the BSO because of the regulatory aspects that must be included. An example of mandated general biosafety training is initial bloodborne pathogens training and annual retraining.

Mandated general biosafety training is required for all laboratory workers (faculty, staff, students, and visiting scientists) at MSU. The exact training required for a particular person will depend on the hazards to which he or she is exposed. “Biosafety Training for Laboratory Workers” is a training program offered by Biosafety Officer that is designed for those working in laboratories.

New employees, faculty, and staff must attend this training program immediately following their hiring or as soon as practical and before beginning laboratory work. Attendance at new employee orientation does not fulfill this requirement. Training includes, but is not limited to, laboratory safety practices, biosafety, bloodborne pathogens, and hazardous waste operations.
Biosafety Training for Laboratory Workers

Laboratory safety training satisfies the basic competency regulatory requirements for those working in labs. It does not satisfy the need for department-specific training, shipment of infectious agents, select agents, Biosafety Level 3 work, or other specialized training.

Mandated Specific Training

Mandated specific training is also required by law and/or policy. In some cases, it is administered and tracked by the Biosafety Officer, which maintains the record files. Examples of mandated specific training include, agent specific trainings, or other specific training required by the IBC. Individuals working in laboratories classified as BSL-3, or who are potentially exposed to specific zoonotic diseases, must also undergo training.

Training laboratory personnel in the unique hazards, equipment, and procedures for a given laboratory is the responsibility of the PI or laboratory manager to administer, document, and track. This training is mandated and must be provided by the PI or laboratory manager on a periodic basis to all laboratory personnel. Documentation is also required and must include at least the date and duration of training, name and position of the trainer, topics covered, and names of the trainees.

Packaging and Shipping of Infectious Agents Training

Personnel who package and ship infectious agents and diagnostic specimens such as microorganisms, blood samples, and clinical samples for pathological testing are required by federal and international regulations to receive training every two years. Biosafety Office offers this training periodically and upon request.

Select Agents Training

Personnel authorized to use select agents are required to receive training. This training is designed to meet the specific requirements of the 42 CFR 73 requirements and must be completed prior to any individual starting work with select agents; in addition there is an annual refresher course that all authorized individuals must attend in order to continue with their ability to work with select agents. The RO and the lab will maintain copies of the training records for reference.

In addition, personnel authorized by the IBC to work with specific agents designated as biological agents with the potential to cause LAI are also required to receive agent specific training.

Contact the BSO for more information.
**Biosafety Level 3 Training**

Specialized BSL-3 training is required for individuals who work in a BSL-3 containment lab or in the BSL-3 biocontainment facility.

**Laboratory-Specific Training**

Individual laboratories are required to develop specific training for the particular agents and procedures that personnel will perform in that laboratory. This training should be specific to the hazards in the laboratory and to each person’s laboratory duties. Each person in the laboratory must understand the hazards associated with the agent and laboratory operations, how to prevent exposures to biological and chemical agents (see Chemical Hygiene Plans), and trained on the laboratory standard operating procedures. Laboratory-specific training should not duplicate the general biosafety training, but instead should supplement it.

Each laboratory must maintain training records. The records should include the names of personnel in the laboratory and their most recent dates of Laboratory Safety Training and training provided specifically by the lab PI or supervisor. Records are also updated and maintained in the Biosafety and Chemical Safety Logbooks. The information will be updated and maintained by the PI or supervisor. Ongoing training is required as new hazards and procedures are introduced into the laboratory. The occurrence of spills, spread of contamination, near misses, etc., also indicate the need for refresher training.

**Other Safety Training**

Personnel who utilize hazardous chemicals, radioisotopes, or x-ray generating devices must attend additional laboratory safety trainings.

**Refresher Training**

All laboratory workers and certain categories of building occupants will be subject to periodic mandatory refresher training. The scope and details of these refresher trainings will be determined by the IBC and will range from annually (for those required by regulatory mandates, such as Bloodborne Pathogen Standard or Select Agents Rule) to every three years.
Chapter 12 - Decontamination and Sterilization

Decontamination is a process or treatment that renders a device, instrument, or work surface safe to handle. A decontamination procedure can range from sterilization by autoclave or ethylene oxide to simple cleaning with soap and water. Sterilization, disinfection, and antisepsis are all forms of decontamination.

Sterilization is the use of a physical or chemical procedure to destroy all microbial life, including highly resistant bacterial endospores.

Disinfection eliminates virtually all pathogenic, non-spore-forming microorganisms but not necessarily all microbial forms on inanimate objects (work surfaces, equipment, etc.). Effectiveness is influenced by the kinds and numbers of organisms, the amount of organic matter, and the object to be disinfected and chemical exposure time, temperature, and concentration.

Antisepsis is the application of a liquid antimicrobial chemical to skin or living tissue to inhibit or destroy microorganisms. It includes using germicidal solutions for swabbing an injection site on a person or animal and for handwashing. Although some chemicals may be utilized as either a disinfectant or an antiseptic, adequacy for one application does not guarantee adequacy for another. Manufacturers’ recommendations for appropriate use of germicides should always be followed.

General Procedures

Decontamination of cultures and objects contaminated by biological agents is routinely performed in laboratories. Decontamination is a vital component of microbiological safety practice and serves to protect laboratory personnel (as well as others) from infection and the release of infectious organisms to the outside environment (primarily through person-to-person transmission). Decontamination of media, work surfaces, and equipment is also necessary to prevent contamination of cultured organisms.

Infectious wastes such as liquid and solid will be handled, treated and disposed according to hazardous waste policies and procedures. Liquid wastes such as bacterial or viral culture media from BSL2 labs will be treated with appropriate disinfectant prior to sink disposal. Solid wastes from the BSL2 laboratories will be segregated and placed in biohazard containers lined with biohazardous waste bags and disposed as biological wastes.

- Autoclaving is the preferred method for treating biological wastes.
- A disinfectant should be chosen that is appropriate for the organism in use.
- All liquid biological cultures should be deactivated with appropriate disinfectant.
- All solid biological waste should be disposed of in the biohazard waste containers.
Methods of Decontamination

The three main categories of physical and chemical decontamination are heat, liquid disinfection, and vapors and gases.

- **Heat:** Wet heat is the most dependable method of sterilization. Autoclaving (saturated steam under pressure of approximately 15 psi to achieve a chamber temperature of at least 250° F for a prescribed time) is the best method of rapidly achieving destruction of all forms of microbial life.
- **In addition to proper temperature and time, prevention of entrapped air is critical to achieving sterility because of air’s poor heat transfer properties.**
- **Material to be sterilized must come into contact with steam and heat. Indicators of proper autoclave operation (e.g., autoclave tape or autoclave-sensitive labels) must be used with each load to visually indicate successful processing.**
- **Use of autoclave tape alone is not an adequate monitor of the sterilization’s success.**
- **Liquid disinfection:** A liquid disinfectant (e.g., 1:10 solution of household bleach yielding a final hypochlorite concentration of 0.5%) is used to wipe or soak potentially contaminated materials for a period of time to kill all pathogenic agents present. Each disinfectant requires varying amounts of contact time.
- **Gas and vapor:** Potentially contaminated articles are exposed to a sterilizing gas (e.g., ethylene oxide, or ETO) or vapors from a chemical (e.g., formaldehyde). Because of the hazardous nature of the gases and vapors used, this requires specially designed equipment and facilities.

**Autoclaving**

Autoclaving uses saturated steam under pressure (approximately 15 psi) to achieve a temperature in the autoclave of at least 121° C (250° F). Autoclaving can be used to destroy vegetative bacteria, bacterial spores, and viruses. When decontaminating biohazardous waste, it is recommended that the temperature in the waste reach a minimum of 120° C for a minimum of 30 minutes. The total processing time required to meet these conditions depends on several loading factors (see below); however, it is recommended that a minimum autoclave cycle of one hour be used when decontaminating waste.

When using an autoclave, the following guidelines should be taken into consideration:

- **Biohazardous materials should not be placed in autoclaves overnight in anticipation of autoclaving the next day.**
- **Autoclaves should not be operated by untrained personnel.**
- **Special precautions should be taken to prevent accidental removal of material from an autoclave before it has been sterilized or the simultaneous opening of both doors on a double door autoclave.**
• Dry hypochlorite, or any other strong oxidizing material, must not be autoclaved with organic materials such as paper, cloth, or oil:

Temperature: an autoclave uses steam under a pressure of approximately 15 psi to achieve a chamber temperature of at least 121° C. Although the autoclave chamber may reach 121° C, this does not necessarily mean that the interior of the load will reach this temperature.

Time: a minimum autoclave cycle time of 20 minutes at a chamber temperature of 121° C (time does not begin as soon as the autoclave cycle is initiated) is commonly recommended for sterilization of clean items. However, the total processing time required to achieve decontamination depends on several loading factors, including the load container (heat transfer properties); the amount of water added to the load; and the weight of the load. For increased loads, an increased cycle time will be required to ensure effective decontamination.

Contact: steam saturation is essential for maximum heat transfer. Steam must contact all areas of the load. Autoclave bags and other containers should be left partially open (or otherwise permit entry of steam) to ensure adequate contact. Studies have shown that adding water to the interior of the bag improves the time-temperature profile of the autoclave cycle, thereby increasing the autoclave’s sterilization efficiency.

**Dry Heat**

Requiring higher temperature and longer contact time, dry heat is less effective than moist heat (autoclaving). Nevertheless, dry heat is preferable to moist heat for decontamination of anhydrous materials and closed containers because the moisture component of the steam used in an autoclave will not effectively penetrate anhydrous materials and closed containers.

The highest dry heat equivalent temperature that these materials will reach in an autoclave is 121° C. The highest temperature that material will reach in a dry heat oven will be the actual temperature inside the oven. A temperature of 160°-180° C for three to four hours is recommended for decontamination of waste using a dry heat oven.

**Chemical Disinfection**

Disinfection is the decontamination of work surfaces, equipment, biological safety cabinets, and other inanimate objects using antimicrobial agents. Several chemical agents are used as disinfectants.

Laboratory workers should remember that there are hazards associated with all of these chemical disinfectants.

• Inhalation and skin contact should be minimized, and eye contact avoided.
• Appropriate gloves and safety eyewear should always be worn when handling these chemicals.

Pertinent information for some of the common chemical disinfectants is summarized in table format at the end of this chapter.
<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>Use Parameters</th>
<th>Effective Againsta</th>
<th>Important Characteristics</th>
<th>Potential Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol (ethyl, isopropyl)</td>
<td>conc.: 70-85%</td>
<td>+</td>
<td>Eye irritant, toxic, flammable, inactivated by organic matter.</td>
<td>Surfaces: work and equipment</td>
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<td></td>
<td>contact time: 10-30 min.</td>
<td>+</td>
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<tr>
<td>Chlorine Compounds</td>
<td>conc.: 0.05-0.5% (commercial bleach 0.5%)</td>
<td>+</td>
<td>May leave residue; corrosive; skin, eye and respiratory irritant; inactivated by organic matter; make up at least weekly.</td>
<td>Spills, equipment surfaces, instruments, glassware, water baths</td>
</tr>
<tr>
<td></td>
<td>contact time: 10-30 min.</td>
<td>+</td>
<td></td>
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<tr>
<td>Quaternary Ammonium Compounds</td>
<td>conc.: 0.1-2%</td>
<td>+</td>
<td>Toxic, inactivated by organic matter.</td>
<td>Surfaces (work and equipment), BSCs, floor maintenance, glassware, instruments</td>
</tr>
<tr>
<td></td>
<td>contact time: 10-30 min.</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenolic Compounds</td>
<td>conc.: 0.2-3%</td>
<td>+</td>
<td>Leaves residue; corrosive; skin, eye and respiratory irritant; toxic; inactivated by organic matter.</td>
<td>Surfaces (work and equipment), BSCs, floors, spills, glassware, instruments, water baths</td>
</tr>
<tr>
<td></td>
<td>contact time: 10-30 min.</td>
<td>+</td>
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<tr>
<td>Iodophor Compounds</td>
<td>conc.: 0.47%</td>
<td>+</td>
<td>Leaves residue; corrosive; skin and eye irritant; toxic; inactivated by organic matter.</td>
<td>Surfaces (work and equipment), BSCs, glassware, water baths</td>
</tr>
<tr>
<td></td>
<td>contact time: 10-30 min.</td>
<td>+</td>
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<tr>
<td>Formaldehydeb</td>
<td>conc.: 4-8%</td>
<td>+</td>
<td>Leaves residue; skin, eye</td>
<td>Less effective than other</td>
</tr>
</tbody>
</table>

a:  += very positive response, ± = less positive response. A blank denotes a negative response or not applicable.
b: due to its irritating characteristics and status as a carcinogen, formaldehyde should not be used without good local exhaust ventilation.

Chapter 13 - Biohazardous Spill Response

Even with the most careful planning and implementation of a research project, the possibility of an incident or spill involving biological materials exists. The following procedures are intended to provide a planned response to such rare events.

In any spill scenario, the priority of actions is determined by the “PEP” rule - People, Environment and Property. The highest priority is to provide aid to injured personnel and prevent spill area access to others.

Preplanning for Biohazardous Spill Cleanup

All spills of biohazardous materials do not represent the same risk to personnel and the environment, making each spill somewhat unique. The volume of a spill is not necessarily a valid measure of the risks involved. For example, dropping a glass vial containing 1.0 ml of lyophilized anthrax spores poses much greater risk to laboratory staff than dropping a 10 liter glass bottle of Escherichia coli K-12 culture.

Factors other than volume that must be considered in spill risk assessment include:

- Location (e.g., biohazard cabinet, countertop, floor, equipment)
- Nature (e.g., tip-over, aerosolizing (spray/splash), drop from a height)
- Toxicity/infectivity of spilled material
- Volatility and viscosity of spilled material
- Other properties of material (e.g., pH, normality, temperature)
- Nature of affected surfaces (e.g., absorbent, pitted, smooth)
- Complicating materials (e.g., broken glass, clothing, mixing with other materials)
- Susceptibility of spilled material to neutralization/disinfection

Nevertheless, preplanning of spill response will lower the risk of cleaning up a spill and will increase the likelihood that the spill is handled appropriately. Principal Investigators or Laboratory Directors should prepare their laboratory for typical spill scenarios expected in the laboratory. Laboratory workers should be informed of the hazards of the biological agents used in the laboratory, the risk associated with these agents during spill scenarios, how to safely clean up the agents, and how to properly dispose of cleanup materials.
**Spill Cleanup Materials**

Each laboratory area should have spill cleanup materials available to respond to the largest spill anticipated for that area. At a minimum, the following spill cleanup materials should be available in the laboratory:

- Gloves (thick, chemical-resistant gloves or double pair of thin, nitrile gloves are recommended)
- Safety goggles and masks or a face shield (strongly recommended to avoid splashes to the nose and mouth)
- Lab coat or smock to protect clothing and body
- Absorbent pads
- Disinfectant appropriate for the agents used in the laboratory
- Forceps or other devices to pick up contaminated material (especially sharps)
- Sharps disposal container
- Autoclavable biohazard bags

The spill kits distributed by Safety and Risk to MSU laboratories may not be adequate for the response to a biological spill.

**Biohazardous Spill Cleanup Risk Assessment**

Several factors must be considered when assessing the risk that a spill represents:

- Volume and concentration of the spilled material
- The infectious dose of the spilled material and routes of exposure
- Location of the spill
- Degree of aerosolization of the agent resulting from the spill
- Susceptibility of the spilled material to disinfection
- Nature of the affected surface(s) and its ability to “hide” organisms from disinfection
- Immune status of immediate personnel

As with any spill scenario (biological, chemical, or radiological), the safety of personnel is the most important consideration. Cleanup is to begin only after it is determined that the personnel who will clean up the spill have appropriate knowledge, training, and equipment.
Biohazardous Spill Cleanup Procedures

The following are general biohazardous spill cleanup procedures that are appropriate for most spill scenarios; however, the appropriate response to any spill is based on an assessment of the risk associated with that particular situation.

If in doubt, immediately call the Biosafety Officer at (406) 994-6998.

Biohazardous Spills Inside Biological Safety Cabinets

- Wear a laboratory coat (disposable recommended), safety glasses, and gloves (appropriate for the biological agent and the chemical disinfectant) during cleanup.
- Allow the BSC to run continually during cleanup.
- Surround the affected spill area with absorbent material to prevent spread of the spill.
- Apply disinfectant appropriate for the biological agent and allow a minimum of 20 minutes contact time (or as directed by manufacturer’s instructions). Alcohol or other flammable liquids are not recommended.
- Wipe up the spill with a disposable cloth or a towel soaked with disinfectant.
- Wipe the BSC’s walls and work surface, as well as any equipment in the cabinet, with a disinfectant- soaked cloth.
- Place contaminated items in an appropriate container (biohazard waste bag, sharps container, or autoclavable pan with lid for reusable items) for autoclaving.
- Remove protective clothing and place in a biohazard waste bag for autoclaving.
- Thoroughly wash hands and forearms with soap and water.
- Allow BSC to run for a minimum of 10 minutes before resuming work in the cabinet or shutting off the cabinet.

Biohazardous Spills in the Laboratory, Outside the Biological Safety Cabinet

If a BSL-1 agent or less than 100 ml of a BSL-2 agent is spilled, the following procedures should be followed:

- Remove any contaminated clothing and place in a biohazard waste bag for autoclaving, and wash all areas affected by skin contact with soap and water.
- Wear a long-sleeved gown or lab coat (disposable recommended), shoe covers, safety glasses (face shield also recommended), and gloves (appropriate for biological agent and disinfectant).
• Place absorbent pads over the spill (to absorb liquid), then place a second layer of disinfectant-soaked absorbent pads over the spill.

• Pour additional disinfectant around the spill, being careful to minimize aerosolization, and work from the periphery toward the center, ensuring thorough contact between the spill and the disinfectant. Disinfect all items in the spill area.

• Allow a minimum of 20 minutes contact time (or as directed by manufacturer’s directions) with the disinfectant.

• Wipe down all equipment, tools, etc., with disinfectant.

• Place contaminated items in an appropriate container (biohazard waste bag, sharps container, or autoclavable pan with lid for reusable items) for autoclaving.

• Remove protective clothing and place in a biohazard waste bag for autoclaving.

• Thoroughly wash hands, forearms, and face with soap and water. It is recommended that cleanup personnel shower as soon as possible.

If the spill involves a BSL-3 agent, or greater than 100 ml of a BSL-2 agent, immediately evacuate all personnel from the affected area. Wait for aerosol to settle (usually a minimum of 30 minutes) before entering the spill area. Exception: If the laboratory is not under negative pressure, cleanup should begin as soon as possible to minimize the spread of aerosols.

In addition, the following procedures should be followed:

• Remove any contaminated clothing and place in a biohazard waste bag for autoclaving and wash all areas affected by skin contact with soap and water.

• Wear a long-sleeved gown or lab coat (disposable recommended), shoe covers, safety glasses (face shield also recommended), and gloves (appropriate for biological agent and disinfectant). For cleanup of a BSL-3 agent, a HEPA-filtered respirator may be required.

• Place absorbent pads over the spill (to absorb liquid), then place a second layer of disinfectant-soaked absorbent pads over the spill.

• Pour additional disinfectant around the spill, being careful to minimize aerosolization, and work from the periphery toward the center, ensuring thorough contact between the spill and the disinfectant.

**Disinfect all items in the spill area.**

• Allow a minimum of 20 minutes contact time (or as directed by manufacturer’s directions) with the disinfectant.

• Wipe down all equipment, tools, etc., with disinfectant.

• Place contaminated items in an appropriate container (biohazard waste bag, sharps container, or autoclavable pan with lid for reusable items) for autoclaving.

• Remove protective clothing and place in a biohazard waste bag for autoclaving.
• Thoroughly wash hands, forearms, and face with soap and water. It is recommended that cleanup personnel shower as soon as possible.

Biohazardous Spills Inside a Centrifuge

• Clear the area of all personnel and allow aerosol to settle (usually a minimum of 30 minutes) before re-entering the area.
• Wear a laboratory coat (disposable recommended), safety glasses, and gloves during cleanup. For a BSL-3 agent, a HEPA-filtered respirator may be required.
• Transfer the rotor and buckets to a BSC for cleanup.
• Using an appropriate disinfectant, thoroughly disinfect the inside of the centrifuge, the rotor, and buckets.
• Discard cleanup materials and protective clothing as biohazardous waste.
• Thoroughly wash hands, forearms, and other parts of the body with soap and water.

Biohazardous Spills Outside the Laboratory During Transport

All biological agents are to be transported from the laboratory inside an unbreakable, well-sealed, primary container containing absorbent material that is contained inside a second unbreakable, well-sealed leak-proof container (see Chapter 11 for transportation guidelines). Both the primary and secondary containers must be labeled with the universal biohazard symbol and the identity of the agent. In the event a transport container drops and its contents are spilled, the following procedures should be followed:

• Immediately clear the area of all personnel and secure the area.
• Cleanup should be initiated as soon as possible to prevent spread of aerosol. Attempt cleanup only if appropriate cleanup materials and protective clothing are available.
• Notify the Biosafety Officer (406)-994-6998

When responding to a spill, the following rules should be followed:

• Tend the injured: Ensure receipt of immediate medical care and do not attempt to move the injured individual(s) unless ambient conditions become life-threatening. Individuals splashed, sprayed with, or otherwise exposed to human blood or other body fluids or tissues during a spill will need to remove contaminated clothing and utilize basic first aid, washing any wounds immediately.
• Await assistance: Unless laboratory personnel are trained and properly supplied with personal protective equipment, DO NOT attempt to clean up the spill. Personnel should immediately call the Biosafety Office at (406)-994-6998

• Isolate the spill: Evacuate the immediate spill area or the entire room in the case of an aerosolizing (splashing or spraying) spill or a spill of volatile material. Prevent others from entering the spill area with barricades or, if necessary, a sentry.

• Contain the spill: Place absorbent material around, on, or in the flow path of the spilled material only if it can be done safely.

• Provide information: Provide the information requested by the Biosafety Office or Safety and Risk personnel and await arrival of the emergency provider.

• Clean up: Clean up should take place ONLY if laboratory personnel are trained, properly supplied with personal protective equipment, and otherwise able to clean up and disinfect the spill safely.
Chapter 14 - Biohazardous and Medical Waste Disposal

The following types of waste are identified and defined as infectious or physically dangerous medical or biological waste:

- Blood and blood products: Discarded bulk human blood and blood products in free draining, liquid state; body fluids contaminated with visible blood; and materials saturated/dripping with blood.
- Pathological waste: Human anatomical parts, organs, tissues, and body fluids removed and discarded during surgery or autopsy, or other medical procedures and specimens of body fluids and their containers.
- Cultures and stocks of infectious agents and associated biologicals: All discarded cultures and stocks of infectious agents and associated biologicals, biotechnological by-product effluents, cultures of specimens from medical and pathological laboratories, cultures and stocks of infectious agents from research laboratories, wastes from the production of biologicals, and discarded live and attenuated vaccines intended for human use.
- Contaminated animal carcasses, body parts and bedding: The contaminated carcasses and body parts and bedding of all research animals known to be exposed to pathogens.
- Sharps: Discarded medical articles that may cause puncture or cuts, including but not limited to all, used and discarded hypodermic needles and syringes, Pasteur pipettes, broken medical glassware, scalpel blades, disposable razors, and suture needles.
- Biotechnological by-product effluents: Any discarded preparations made from genetically altered living organisms and their products. Infectious or physically dangerous medical or biological waste shall be referred to as “Waste” in the subsequent provisions of 105 CMR 480.000.

**Biohazardous Waste**

Biohazardous waste includes waste materials derived from cultures and stocks of infectious agents, human pathological wastes, contaminated and non-contaminated animal carcasses and body parts, all sharps, and human blood and blood products.

Proper handling and disposal of biohazardous waste is necessary to prevent infection of personnel (laboratory workers, custodians, laboratory visitors, etc.) and release to the environment. OSHA and Commonwealth of Massachusetts regulations (105 CMR 480.000) require that biohazardous waste be properly labeled, stored, and disposed of.
Labeling Biohazardous Waste

At a minimum, all biohazardous waste must be labeled with the universal biohazard symbol. Additional information, such as the type of waste (such as “sharps” or “liquid waste”) and origin of the waste (biological agent, lab, PI, and responsible person) is required.

Handling and Disposal of Biohazardous Waste

Sharps

Sharps include all syringes, lancets, scalpels, and other similar medical instruments (whether or not contaminated), as well as contaminated Pasteur pipettes and broken glass, and other instruments or materials that can cut or puncture personnel.

- Sharps must be collected in rigid containers that are leak-proof and resistant to puncture from the sharps. Sharps containers must be designed so that sharps can be safely introduced into the container but not easily retrieved.
- Containers should be red in color and labeled with the universal biohazard symbol. When the sharps container is approximately 3/4 full, MSU personnel should seal the waste container and it will be picked up by the Safety and Risk.

Uncontaminated Laboratory Glassware and Broken Glass

Collect uncontaminated laboratory glassware and broken glass in rigid containers (separate from other waste) that will prevent cuts and punctures to personnel. Containers should be labeled “broken glass.” Broken glass is to be disposed of as ordinary trash.

Solid Biohazardous Waste

Solid biohazardous waste includes microbial agents, tissue culture, and contaminated material (such as petri dishes, pipettes, etc.). These materials are collected in orange or clear biohazard bags that are double-lined and autoclaved.

Liquid Biohazardous Waste

Liquid biohazardous waste includes all blood and liquid waste from humans or animals, and all other liquid biohazardous waste (such as microbial cultures). Collect liquid waste in closeable, rigid, plastic, leak-proof containers labeled with the universal biohazard symbol.
• Human and animal blood and body fluids can be disposed of by flushing directly to the sanitary sewer after chemical disinfection with appropriate contact time. (wear laboratory coat, safety glasses and face shield, and gloves, and be careful to minimize splashing).
• All other liquid waste must be autoclaved or treated with a disinfectant prior to disposal.
• Liquid waste treated with small quantities of bleach or other household disinfectants can be disposed of by flushing directly to the sanitary sewer after sufficient contact time. Liquid waste treated with other chemical disinfectants must be disposed of as hazardous chemical waste through Safety and Risk.

**Animal Carcasses, Body Parts, and Tissue**

• Infectious animal carcasses are placed in a red biohazard bag secured with a plastic tie and incinerated through Safety and Risk.
• All non-preserved carcasses should be stored in a freezer or cold storage area prior to disposal. Secure limbs and sharp protrusions so they do not puncture the bag.
Chapter 15 - Transportation of Biological Materials

The packaging and transportation of biological materials are subject to strict local, state, federal, and international regulations. This is particularly so if the material is transported through the “public domain,” namely, those roadways, airways, and sea lanes accessible to the public.

The intent of the packaging and transportation regulations is to prevent accidental exposure of personnel who may handle the material during its shipment. Therefore, certain general criteria apply to all possible transportation scenarios.

Prior to transporting any biological materials, the following controls must be in place:

- Emergency procedures (e.g., contact names and information, spill cleanup, disinfection protocols, etc.) must be known to the person carrying the materials.
- Container must be appropriate for the material being transported.
- Material must be packed so that it will stay upright during transportation.
- The containers must be properly labeled.
- Proper protective clothing must be worn during the packaging of the material.
- Hands should be washed after handling materials.
- Open cuts or other wounds should be covered before handling the materials.
- Aerosol generation must be avoided when handling and packing the materials.
- The person packaging the material must ensure that the exterior surfaces of each package are free of any potential contamination by the packed material.

Transportation on Campus

The following requirements must be observed during the transportation of biological materials within a campus (e.g., between two laboratories):

- At a minimum, all laboratory materials must be transported in a secondary container that is shatterproof, and leak-proof. Materials should never be carried in hands or pockets.
- The secondary container should be closeable and easy to decontaminate; an absorbent pad (or similar material) should be placed inside the secondary container to absorb any spills.
- A laboratory coat should be worn during transport.
- Label information must include the identity of the biological material or agent, the universal biohazard symbol (if the material or agent is in, or above, Risk Group 2), and the sending and receiving laboratory identification (e.g., PI name and room number).
- Each individual container must have enough label information to identify its contents. Other information should be on the outside of the package.
• The container should be carried directly to the intended laboratory and not taken to offices, cafeterias, or other public or inappropriate locations.
• Upon delivery, the receiving laboratory personnel should be informed and the material properly stored.
• The package should be carefully inspected for signs of leakage or other contamination and, if necessary, decontaminated before opening.

**Packaging and Shipping Infectious Agents via Domestic Flights**

Occasions do arise when a PI must either ship or receive biological materials from another institution. Such activities are governed by strict federal and international guidelines.

The International Civil Aviation Organization (ICAO) is the United Nations entity that governs all international civil aviation matters. The ICAO’s Technical Instructions for the Safe Transport of Dangerous Goods by Air govern the shipping of dangerous goods. These technical instructions have been incorporated into U.S. law and are an acceptable method of transport in the United States (49 CFR 175).

Packaging and shipping biological materials involves certain risks with numerous potential liabilities. The International Air Transport Association’s (IATA) Dangerous Goods Regulations (DGR), latest edition, is the worldwide gold standard for shipping. The IATA regulations apply to all air transport, both domestic and international flights. Following IATA’s DGR ensures that a package will also meet U.S Department of Transportation requirements for ground transport.

All responsibilities for packaging and shipment of these agents have been assigned to the shipper. Only properly trained personnel may offer infectious materials for transport. The following is only a summary of the requirements for packaging and shipping infectious agents and does not constitute proper training.

**Definitions and Applicability**

• Dangerous goods: articles or substances capable of posing significant risk to health, safety, property, or the environment when transported by surface or air. Most infectious or biological materials are considered dangerous goods and therefore subject to shipping regulation.
• Infectious substances: substances known or reasonably expected to contain pathogens. Pathogens are defined as micro-organisms (including bacteria, viruses, rickettsiae, parasites, fungi) and other agents, such as prions, which can cause disease in humans or animals.

For the purposes of shipping classification, infectious substances are broken into two categories:

Category A: an infectious substance transported in a form that, when exposure to it occurs, is capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals.
Category B: an infectious substance that does not meet the criteria for inclusion in Category A.

- Biological products: those products derived from living organisms manufactured and distributed in accordance with the requirements of national governmental authorities (e.g., the FDA). They may have special licensing requirements and are used either for prevention, treatment, or diagnosis of disease in humans or animals, or for developmental, experimental, or investigational purposes related thereto.

Biological products manufactured and packaged in accordance with the requirements of appropriate national authorities; transported for the purposes of final packaging or distribution; and for personal health-care use by medical professionals are NOT subject to dangerous goods regulation. However, biological products not governed by national authorities and that are known or reasonably believed to contain infectious substances MUST be classified and shipped according to dangerous goods regulations.

Exempt Patient Specimens: patient specimens for which there is a minimal likelihood that pathogens are present are exempt from most of the shipping regulations. However, they must be marked with the words “exempt human specimen” or “exempt animal specimen” and must be triple-packed as described below.

Completely Exempt Substances: materials that are totally exempt for consideration under the shipping regulations:

- Substances containing micro-organisms that are non-pathogenic to humans or animals
- Substances in a form so that any present pathogens have been neutralized or inactivated such that they no longer pose a health risk
- Environmental samples (including food and water samples) that are not considered to pose a significant risk of infection, and
- Dried blood spots, fecal occult blood screening tests, blood or blood products intended for transfusion, and tissues or organs intended for transplantation.
- Classification and Identification

The substance to be shipped must be classified as completely exempt from regulation, an exempt patient specimen, or a Category A or B infectious substance. Once classified, proper shipping names and identification numbers can then be assigned to the material. Exempt patient specimens do not require shipping names and identification numbers. However, Category A and B materials are assigned the following names and numbers:

Category A: assign one of two identifiers, depending on whether or the material infects humans:

- Infectious substance affecting humans: UN 2814
- Infectious substance affecting animals: UN 2900

Note: If a material infects both humans and animals, use the Infectious substance affecting human code, UN 2814.
Category B: biological substance category B: UN 3373

All regulated infectious substances, including Category A, Category B, and exempt patient specimens, must be triple packed:

- The innermost primary receptacle(s) is leak-proof.
- A leak-proof secondary receptacle with absorbent material placed between the primary and secondary receptacles to prevent the release of liquid during transport and to shield multiple primary receptacles from coming in contact with one another.
- Rigid, tertiary outer packaging that is at least 100 mm (4 in) in its smallest external dimension. Additionally, shipments of Category A and Category B materials must be packaged according to IATA Packing Instructions 602 and 650, respectively. Those guidelines require the following:
  - Shipments must be prepared in such a way that they arrive at their destination in good condition and present no hazard to persons or animals during shipment.
  - Outer packaging must meet structural strength requirements and carry defined specification markings.
  - Packages must be at least 100 mm (4 in) in their smallest external dimension.
  - An itemized list of contents must be enclosed between the secondary container(s) and the outer packaging.
  - All packages containing infectious substances must be marked durably and legibly on the outside of the package with the name and telephone number of a person responsible for the shipment.
  - The shipper must make advance arrangements with the recipient and the operator to ensure the shipment can be transported and delivered without unnecessary delay.
  - Substances shipped at ambient temperatures or higher must be in primary receptacles made only of glass, metal or plastic, with a positive means of ensuring a leak-proof seal. Screw caps must be reinforced with adhesive tape.
  - Substances shipped refrigerated or frozen must carry the refrigerant between the secondary container and outer packaging. Wet ice is not recommended for shipping as it may cause the package to leak during transport, thus delaying or causing rejection of the package by the transporter. If dry ice is used, the packaging must permit the release of CO2 gas.
  - Primary and secondary containers must retain their integrity across the full range of pressures and temperatures experienced under normal and loss-of-refrigerant conditions.
Labeling

Package labeling is in the form of standardized pictures that must be affixed to the outside. The color and design of each label is prescribed in the IATA regulations. All labels must be at least 2 inches on the smallest side.

For the purposes of infectious substances, five different labels must be considered:

Category A:

Category B:

Dry Ice:

Cargo Aircraft Only: must be affixed if shipping volumes greater than 50 ml of a Category A substances

Orientation Arrows: if shipping liquids, two such labels must be affixed to the package, on opposing sides.
Marking

Markings are the words and numbers required to be on the outside of a package. The following markings must be present on any package containing a Category A or Category B material:

- **UN Number and Proper Shipping Name:**
  - UN 2814 Infectious substance affecting humans
  - UN 2900 Infectious substance affecting animals
  - Biological substance category B

**Note:** The UN number is part of the label for Category B substances.

- **Contact Information**
  - Name and telephone number of the responsible person
  - 24-hour emergency telephone number in case of transportation emergency
  - “To” and “from” information

If shipping a material under dry ice, the following additional marking is required:

- UN 3373
  - UN 1845 Dry Ice (the weight in kilograms of the dry ice present should also be noted)

If shipping an exempt patient specimen, the only marking required is:

- Exempt Human Specimen
  - or
  - Exempt Animal Specimen

Training Requirements

Those involved in the packaging and shipping of infectious substances must undergo training every two years or when activities change. It is the department’s responsibility to ensure training is completed. The shipper is obligated to receive further qualification when shipping hazardous materials of a class or division where current training is insufficient.

Shipping Documents

Shipping papers describing the material in transit must accompany all shipments of dangerous goods. For ground transport, a Bill of Lading is required. For air transport, an Airway Bill takes the place of a Bill of
Lading. However, air transport of a Category A material also requires that a *Shipper’s Declaration of Dangerous Goods* be filled out. The full and accurate completion of the Shipper’s Declaration is essential, as these are legal documents signed by the shipper, which creates a contract between the shipper and the carrier. The document must be accurate, legible, and neat and without any spelling errors.

- The declaration form must be completed in English.
- Three copies of the declaration must be completed. One copy will remain with the shipper (PI). Two copies will be sent with the shipment. If the declaration is not a three-part NCR form, photocopies must be made.

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<th>INSTRUCTIONS</th>
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| 1 | **Shipper’s:**
|   | ♦ Name
|   | ♦ Address
|   | ♦ Phone number |
| 2 | **Receiver’s:**
|   | ♦ Name
|   | ♦ Address
|   | ♦ Phone number |
| 3 | **Line out the item that does not apply.** Passenger aircraft can only be used to ship quantities less than 50 ml. Cargo aircraft must be used to ship quantities between 50 ml and 4 L. |
| 4 | **Line out the item that does not apply.** |
| 5 | ♦ Proper Shipping Name (infectious substance, affecting humans or infectious substance, affecting animals)
|   | ♦ Identify the specimen by name in parenthesis
|   | *ex. Infectious substance, affecting humans (rabies virus)* |
| 6 | **Class or Division** * Always 6.2 |
| 7 | **UN Code** * UN 2814 or UN 2900 (UN 3373 does not require shippers dec.)* |
| 8 | **Packaging Group** * There is no packaging group for biological agents.* |
| 9 | ♦ Identify by stating the number of containers by the quantity in each container. (e.g., 5 X 10ml)
|   | ♦ Identify type of outer container for the shipment |
| 10 | **Packaging Instructions** * 602 or 650 (also 904 if dry ice included)* |
| 11 | ♦ 24-hour emergency contact number for the shipper (PI, Lab Supervisor),
|   | ♦ The statements, “Prior arrangements as required by the IATA Dangerous Goods Regulations 1.3.3.1 have been made.” And “Prepared according to ICAO/IATA.” |
| 12 | **Name and Signature of the shipper.** |
Appendix A - Importation and Exportation of Etiologic Agents

Multidisciplinary and multi-institutional research is a common practice that involves collaboration among faculty from various institutions and countries. At times it is necessary to share biological samples or materials with collaborators. Federal regulations strictly control the importation and exportation of etiologic agents. The following outlines two major requirements that must be followed.

Note: All importation and exportation of etiologic agents must be processed through the Biosafety Program.

**CDC Etiologic Agent Import Permit Program**

Etiologic agents are those microorganisms and microbial toxins that cause disease in humans and include bacteria, bacterial toxins, viruses, fungi, rickettsiae, protozoans, and parasites. These disease-causing microorganisms may also be referred to as infectious agents. Arthropods and other organisms that transmit pathogens to animals (including humans) are called vectors.

Etiologic agents, vectors, and materials containing etiologic agents are recognized as hazardous materials. Materials containing etiologic agents are regularly transported from one location to another by common land and air carriers. Materials containing etiologic agents must be appropriately packaged to prevent breakage or leakage in order to avoid exposing the package contents to package handlers, transporters, and the general public. Materials containing etiologic agents must be packaged, labeled, and transported in accordance with all applicable regulations. Material containing etiologic agents being imported into the United States must be accompanied by a U.S. Public Health Service importation permit.

**Importation Permits**

Importation permits are issued only to the importer, who must be located in the United States. The importation permit, with the proper packaging and labeling, will expedite clearance of the package of infectious materials through the U.S. Public Health Service Division of Quarantine and release by U.S. Customs.

The importer is legally responsible for ensuring that the foreign personnel package, label, and ship the infectious materials according to federal and international regulations. Shipping labels with the universal biohazard symbol, the importer’s address, the permit number, and the expiration date are also issued to the importer with the permit. The importer must send the labels and one or more copies of the permit to the shipper. The permit and labels inform the U.S. Customs Service and U.S. Division of Quarantine personnel of the package contents.
Federal Regulation

The importation of etiologic agents is governed by the following federal regulation: USPHS 42 CFR - Part 71 Foreign Quarantine. Part 71.54 Etiologic agents, hosts, and vectors.

- A person may not import into the United States, nor distribute after importation, any etiologic agent or any arthropod or other animal host or vector of human disease, or any exotic living arthropod or other animal capable of being a host or vector of human disease unless accompanied by a permit issued by the Director.
- Any import coming within the provisions of this section will not be released from custody prior to receipt by the District Director of U.S. Customs Service of a permit issued by the Director (Centers for Disease Control and Prevention).

Items Requiring Permits

Etiologic agents

It is impractical to list all etiologic agents in this document. In general, an import permit is needed for any infectious agent known or suspected to cause disease in humans.

Biological materials

Unsterilized specimens of human and animal tissues (such as blood, body discharges, fluids, excretions, or similar material) containing an infectious or etiologic agent require a permit in order to be imported.

Hosts and vectors

- Animals: any animal known or suspected of being infected with an organism capable of causing disease in humans may require a permit issued by CDC. Importation of live turtles of less than 4 inches in shell length and live nonhuman primates is regulated by the CDC’s Division of Global Migration and Quarantine (http://www.cdc.gov/ncidod/dq/).
- Bats: all live bats require an import permit from the CDC and the U.S. Department of Interior, Fish and Wildlife Services. The application for a CDC import permit for live exotic bats is at CDC Importation of Animals website (http://www.cdc.gov/ncidod/dq/).
- Arthropods: any living insect or other arthropod that is known or suspected of containing an etiologic agent (human pathogen) requires a CDC import permit.
- Snails: snail species capable of transmitting a human pathogen require a CDC permit.

Packaging Requirements

Infectious materials imported into this country must be packaged to withstand breakage and leakage of
contents and be labeled, as specified in the following federal regulations:

- USPHS 42 CFR Part 72 - Interstate Shipment of Etiologic Agents
- DOT 49 CFR PART 173 - Transportation of Etiologic Agents
- For international shipments, the International Air Transport Association’s (IATA) Dangerous Goods Regulations should be consulted.

Other Permits

- USDA and APHIS permits are required for infectious agents of livestock and biological materials containing animal material. Tissue culture materials and suspensions of cell culture-grown viruses or other etiologic agents containing growth stimulants of bovine or other livestock origins are controlled by the USDA because of the potential risk of introduction of exotic animal diseases into the United States. For more information, contact USDA/APHIS at their website, http://www.aphis.usda.gov/animal_health/.

Principal Investigators must submit USDA/APHIS permit applications via the IBC Office and additional information may be found at http://www.bu.edu/orccommittees/ibc/policies/usdaaphis-permit-application-procedure/.

- U.S. Fish and Wildlife Service permits are required for certain live animals, including bats. For more information, call (800) 344-WILD or visit their website, http://www.fws.gov/.
- Individuals wishing to import select agents and toxins must be registered with the CDC’s Select Agent Program in accordance with 42 CFR Part 73 (Possession, Use, and Transfer of Select Agents and Toxins; Interim Final Rule) for the select agent(s) and toxin(s) listed on the import permit application. Also, in accordance with 42 CFR Part 73.16(a), an APHIS/CDC Form 2 must be completed and submitted to the CDC Select Agent Program and granted approval prior to the shipment of the select agents or toxins under the import permit. Additional information can be found at http://www.cdc.gov/od/sap.

Exportation of Infectious Materials

The export of a wide variety of etiologic agents of human, plant, and animal diseases may require a license from the Department of Commerce. To determine if a license is necessary, visit http://www.bis.doc.gov/Licensing/.

Export control regulations are rather complex and may have multi-agency jurisdictions that must approve activities. The Department of Commerce regulations state that:

“Activities subject to the Export Administration Regulations (EAR) may also be controlled under export-related programs administered by other agencies. Items and activities subject to the EAR are not necessarily exempted from the control programs of other agencies.”

Please contact the Biosafety Program as soon as possible if you intend to export any biological materials.
Appendix B - Laboratory Ventilation and Containment for Biosafety

**Laboratory-ventilated containment equipment fall into three major categories:**

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<thead>
<tr>
<th></th>
<th>Personnel</th>
<th>Product</th>
<th>Environmental</th>
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<tbody>
<tr>
<td>Chemical Fume Hoods</td>
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<tr>
<td>Laminar Flow Clean Benches</td>
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<tr>
<td>Class I Biological Safety Cabinets</td>
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<tr>
<td>Class II Biological Safety Cabinets</td>
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</table>

**Laboratory Chemical ("Fume") Hoods**

Traditional laboratory chemical (or fume) hoods are designed to capture and control chemical vapors and pull them away from the worker. Although the inward flow of air protects the user, chemical hoods do not protect the product (the desired organism being manipulated).

**Horizontal Laminar Flow Clean Bench**

With horizontal laminar flow clean benches, HEPA-filtered air flows horizontally across the workspace.
directly toward the user. These clean benches provide product protection and were originally designed to provide a particulate-free environment for the manufacture of semiconductor components.

Clean benches provide product protection against microbial contamination, but they do not provide personal or environmental protection. In fact, the horizontal flow of air will blow biological agents directly toward the user and into the laboratory. Clean benches are not a biological safety cabinet, and they should not be used with any materials (biological, chemical, or radiological) requiring containment for protection of personnel or the environment.

Clean benches are acceptable for tissue culture work only with cell lines considered to represent low risk (BSL-1 agents) to laboratory workers (including immunocompromised individuals who may frequent the lab). Human cell lines and nonhuman primate cell lines are generally considered to be BSL-2 agents and would not be suitable for use in a clean bench.

Biological Safety Cabinets

Biological safety cabinets (BSCs) are divided into Class I, II, and III (see schematic below). Class II BSCs are subdivided into type A and type B. All BSCs provide personnel and environmental protection, with Class II BSCs also providing product protection.

- Personnel protection is achieved by inward airflow through the front of the cabinet.
- Product protection is achieved by downward HEPA-filtered airflow from the top of the cabinet.
- Environmental protection is achieved by HEPA filtration of exhaust air.
Class I BSC

**Environmental Protection**
Recirculated air going back into the laboratory is HEPA filtered protecting the lab environment.

**Personal Protection**
Directional airflow into the BSC preventing pathogen from coming out.

Class II BSC

**Environmental Protection**
Recirculated air going back into the laboratory is HEPA filtered protecting the lab environment.

**Personal Protection**
Directional airflow into the BSC preventing pathogen from coming out.

**Product Protection**
HEPA filtered laminar downflow and directional downward airflow through the front grills.
<table>
<thead>
<tr>
<th>New NSF Classification, Adopted 2002</th>
<th>Previous NSF Classification</th>
<th>General Description</th>
</tr>
</thead>
</table>
| A1                                    | Class II, Type A             | - 70% air recirculated; 30% exhausted from a common plenum to the room;  
                                           - 75FPM intake;  
                                           - may have biologically contaminated positive pressure plenum |
| A2                                    | Class II, Type A/B3          | - 70% air recirculated; 30% exhausted from a common plenum to the room;  
                                           - 100FPM intake;  
                                           - biologically contaminated plenum under negative pressure or surrounded by negative pressure |
| A2                                    | Class II, Type B3            | - 70% air recirculated; 30% exhausted from a common plenum to a facility exhaust system;  
                                           - 100FPM intake;  
                                           - biologically contaminated plenum under negative pressure or surrounded by negative pressure |
| B1                                    | Class II, Type B1            | - 40% air recirculated; 60% exhausted from cabinet;  
                                           - exhaust air pulled through dedicated exhaust duct into facility exhaust system;  
                                           - 100FPM intake  
                                           - all biologically contaminated plenums are negative to the room or surrounded by negative pressure plenums |
Certification of BSCs

Generally, commercial BSCs are tested by the cabinet manufacturer in accordance with National Sanitation Foundation (NSF) criteria. Cabinets that meet the NSF criteria for performance characteristics, including biological containment, ventilation, cabinet leakage, and HEPA filter leakage, are NSF certified.

Field certification of BSCs is also required to ensure that the cabinet still performs as it did when it obtained NSF certification at the factory. NIH requires field certification under the following circumstances: (1) upon installation of a new BSC; (2) annually thereafter; (3) after repair or maintenance is performed; and (4) after the BSC is relocated.

CDC recommends that BSCs be recertified annually to ensure for proper function. They will also be recertified after being moved to ensure that they have not been damaged. Laboratories are responsible for ensuring that the BSCs are recertified in a timely manner. The contact information to reach the contractor is indicated on the certification sticker affixed on the front of the BSC.

NSF standard 49 provides criteria for construction of BSCs, testing by manufacturers (including biological containment testing), and field certification. NSF has also established a certification program for field certifiers to ensure a minimum level of competency and professionalism. It is recommended that NSF field certifiers be used for field certification of BSCs. Field certification tests include:

Primary tests (BSC performance):

a. Inflow test
b. Down-flow test
c. Smoke pattern test
d. HEPA filter leakage
e. Cabinet leakage (when BSC is newly installed, relocated, or maintenance has been performed that involved removal of access panels)

Additional tests (worker comfort and safety):

a. Noise
b. Vibration
c. Lighting
d. Electrical leakage, polarity, and ground circuit resistance
Appendix C - Autoclave Quality Assurance Program

Autoclaving is an accepted procedure for the decontamination of certain biohazardous waste. Biological cultures, stocks, contaminated solid waste, and liquid waste can be sterilized through autoclaving. After sterilization in a steam autoclave, these materials are considered non-infectious. Materials that contain hazardous chemicals are not to be autoclaved.

To ensure that biohazardous waste is properly decontaminated during autoclaving, the following procedures should be followed by laboratory personnel:

1. Infectious waste must be treated in an autoclave for a minimum of 30 minutes at 121°C (250°F); however, the total processing time required to decontaminate biohazardous waste depends on the specific loading factors (container type, water content, quantity, etc.).
   a. Sterilization by autoclaving is accomplished through exposure and penetration of the contaminated material by superheated steam for an adequate amount of time. Because steam will not penetrate a sealed plastic autoclave bag, bags containing dry loads must not be tightly sealed. To help ensure proper sterilization water may be added to the load. Consult the manufacturer’s instructions for sterilizing materials inside plastic autoclave bags. Liquid waste may also be autoclaved in lieu of adding appropriate chemicals disinfectant, and disposed in the sink.
2. All autoclaved waste must include a steam sterilization indicator (autoclave tape and sterilization indicator strip).
3. Steam autoclaves used to treat infectious waste must operate at a minimum temperature of 121°C. The operating temperature of the autoclave must be verified for each run by maintaining a record of the temperature either as a chart or paper tape recording or a manual recording in a logbook.
4. On a monthly basis (at a minimum), confirm that adequate sterilization conditions are being met, through the use of Biological Indicators (BIs) containing heat-resistant spores (Geobacillus stearothermophilus) placed in the center of autoclave bags (dry loads) or in the liquid (liquid loads) of an autoclave load.
   a. For liquid loads place BIs in container containing water. Remember to use the same type of container that is used for your liquid waste.
   b. There are specific BIs specific for both dry and liquid loads.
5. Maintain records of BI testing and maximum autoclave temperature recordings for a minimum of one year (see Autoclave QC Log at end of appendix).

Monthly Spore Testing Procedure

1. Place Biological Indicator in the center of autoclave bags (dry loads) or in the liquid of an autoclave load.
2. Process the load under normal operating procedures.
3. Incubate the autoclaved BI and a non-autoclaved, control BI according to the manufacturer’s instructions (normally 55°-60°C for 24 to 48 hours).
4. If a color change occurs, the sterilization process was unsuccessful. Discontinue use of the autoclave until it is repaired and passes retesting. Tag the autoclave as “Not Approved for Infectious Waste” until the autoclave passes retesting.
5. Indicate test results on Autoclave QC Log (see end of appendix) and retain for at least one year.
<table>
<thead>
<tr>
<th>Date and time Load Started &amp; users initials</th>
<th>Load Description</th>
<th>Date and time Load Removed &amp; users initials</th>
<th>Lot # &amp; EXP. Date</th>
<th>Results – test ampule after incubation</th>
<th>Results – Control Ampule after Incubation</th>
<th>Comments</th>
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Appendix D - Biosafety Level 2 (BSL-2) Requirements

Biosafety Level 2 (BSL-2) is suitable for experiments involving agents of moderate potential hazard to personnel and the environment.

For example:

- Microorganisms of low biohazard potential, such as those in Risk Group 2 or BSL-2.
- Recombinant DNA activity requiring BSL-2 physical containment including animal studies that involve the construction of transgenic animals.
- Non-recombinant cell and/or tissue culture systems that require this level of containment.
- Oncogenic viral systems classified as low risk.
- Production activities with Risk Group 1 organisms.

The control of potential biohazards at the BSL-2 level is provided by use of standard microbiological practices with the addition of personnel protective equipment (lab coat and gloves).

The following are procedures are used with BSL-2 containment requirements. They are based on the recommendation of the Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition, 2007.

**Standard Microbiological Practices**

- Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.
- Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
- Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.
- Personnel who use contact lenses will consult with EHS if required to use eye protection in the lab.
- Mouth pipetting is prohibited; mechanical pipetting devices are used.
- Policies for the safe handling of sharps are instituted.
- All procedures are performed carefully to minimize the creation of splashes or aerosols.
- Work surfaces are decontaminated upon completion of work, or at the end of the day, and after any spill or splash of viable material with disinfectants that are effective against the agents of concern.
- All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving. Materials to be decontaminated outside the immediate laboratory are placed in a durable, leak-proof container and closed for transport from the laboratory. Materials to be decontaminated off-site are packaged in accordance with applicable local, state, and federal regulations before removal from the facility.
- An insect and rodent control program is in effect.
Special Practices

- Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents is in progress. In general, persons who are at increased risk of acquiring infection, or for whom infection may have serious consequences, are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at increased risk of acquiring infections. The laboratory director in consultation with Research Occupational Health Program has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory or animal room.

- The Principal Investigator or Laboratory Director establishes policies and procedures whereby only persons who have been advised of the potential hazards and meet specific entry requirements (e.g., immunization) may enter the laboratory.

- A biohazard sign must be posted on the entrance to the laboratory when etiologic agents are in use. Appropriate information to be posted includes the agent(s) in use; the biosafety level; the required immunizations; the investigator’s name and telephone number; any personal protective equipment that must be worn in the laboratory; and any procedures required for exiting the laboratory.

- Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).

- Biosafety procedures are incorporated into standard operating procedures or in a biosafety manual adopted or prepared specifically for the laboratory by the laboratory director. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

- The Principal Investigator or Laboratory Director ensures that laboratory and support personnel receive appropriate training about the potential hazards associated with the work involved; the necessary precautions to prevent exposures; and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural or policy changes.

- A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.

- Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.

- Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they 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.

- Syringes that re-sheathe the needle, needleless systems, and other safety devices are used when appropriate.

- Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal according to any local, state, or federal regulations.

- Cultures, tissues, specimens of body fluids, or potentially infectious wastes are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.

- Laboratory equipment and work surfaces should be decontaminated with an effective disinfectant on a routine basis; after work with infectious materials is finished; and especially after overt spills, splashes, or other contamination by infectious materials. Prior to its removal from the facility, contaminated equipment must be decontaminated according to any local, state, or federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations.
• Spills and accidents that result in overt exposures to infectious materials are immediately reported to the Principal Investigator and Laboratory Director. Medical evaluation, surveillance, and treatment are provided as appropriate, and written records are maintained.
• Sinks in the BSL-2 area should be cleared routinely using appropriate disinfectant such as a chlorine-containing abrasive and flushed with a suitable chemical decontaminant.
• Water baths and all water reservoirs should be washed periodically with a suitable chemical decontaminant.
• Once a month, work spaces that do not get daily attention with germicide should be cleaned, as well as other lab areas where clutter accumulates (e.g., storage areas).
• The laboratory will set up a routine schedule to perform surface cleaning with appropriate chemical disinfectant of large equipment (such as incubators) as part of laboratory good practices.
• Supplies should be rotated and outdated material thrown out. Unlabeled material should be eliminated.
• Animals not involved in the work being performed are not permitted in the lab.

Safety Equipment (Primary Barriers)

• Properly maintained biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are to be used when:
• Procedures that have the potential to create infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or embryonate eggs.
• High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a biological safety cabinet.
• Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel.
• Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces, or equipment. Wearing two pairs of gloves may be appropriate. Gloves are disposed of when overtly contaminated and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Disposable gloves are not washed, reused, or used for touching “clean” surfaces (keyboards, telephones, etc.). They should not be worn outside the lab. Alternatives to powdered latex gloves should be available. Hands are washed following removal of gloves.

Procedures for Receiving and Inspecting Samples

• The PI will designate a responsible person for the purchase of all infectious materials to be used in the BSL-2 lab.
• Infectious materials will be shipped to the laboratory in accordance with the appropriate Department of Transportation (DOT) and the International Air Transportation Association (IATA) standards for shipping of infectious biological materials.
• Upon receipt of the package, it will be placed on a tray covered with absorbent material and opened in the Biological Safety Cabinet prevent any potential exposure to personnel in case the container leaked during transport.
• Personnel assigned to open packages will wear lab smock, gloves, and eye protection.
If any containers are found to be damaged, leaking or otherwise contaminated, they will be immediately isolated into a plastic bag along with all packaging materials. The spill will be disinfected and clean up. The Principal Investigator, lab director or designee will be notified immediately. The incident will reported to EHS and as necessary, to appropriate agencies.

If, after inspection, the samples are intact, they can be placed into labeled secondary containers (unbreakable plastic containers or metal tubes) and then transferred to a storage area.

Only staff who are authorized to do so can remove samples from storage. Removal and use of all such materials must be entered into the logbook.

Unused cultures can be returned to storage after the outer container has been properly disinfected.

**Laboratory Facilities (Secondary Barriers)**

In a BSL-2 lab, the following conditions are to exist:

- Lockable doors should be provided for facilities that house restricted areas.
- Consideration should be given to locating new laboratories away from public areas.
- Each laboratory contains a sink for handwashing.
- The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are inappropriate.
- Bench tops are impervious to water and resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.
- Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.
- Biological safety cabinets should be installed in such a manner that fluctuations of the room’s air supply and exhaust air do not cause them to operate outside their parameters for containment. Locate BSCs away from doors, windows that can be opened, heavily traveled laboratory areas, and other potentially disruptive equipment so as to maintain the BSC’s air flow parameters for containment.
- An eyewash station is readily available.
- Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
- There are no specific ventilation requirements. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory. If the laboratory has windows that open to the exterior, they are fitted with fly screens.
Appendix E. MSU Biological Toxin Policy

Biological toxins are toxic substances that can be produced by microorganisms, animals, or plants. Biological toxins are nonreplicating, noninfectious biological materials that can be hazardous even in small quantities.

This document describes Montana State University’s (MSU) policies and relevant Federal regulations that may apply to research with biological toxins. This policy includes Institutional Biosafety Committee (IBC), Federal Select Agent and Export Compliance regulations.

Biological Toxins Requiring IBC Approval

Research at MSU involving biological toxins on the Select Agent list or have an LD$_{50}$ in vertebrates of $\leq$100 ng/kg must be approved by the IBC prior to initiation of work. To determine if the toxin in requires IBC approval, please consult the Toxin Table in Appendix 1 or contact the MSU Biosafety Officer Kirk Lubick @ kirk.lubick@montana.edu. Investigators working with a biological toxin that require IBC approval must complete the IBC protocol form.

Work involving recombinant or synthetic DNAs that encode the active subunit(s) of a biological toxin with an LD$_{50}$ of $\leq$100 $\mu$g/kg in vertebrates, cells, organisms, or viruses must also have IBC approval before initiation of the biological toxin work. In some cases, additional review by the National Institutes of Health Office of Biotechnology Activities (NIH-OBA) as indicated in Section III-B-1 of the NIH guidelines may be required.

Select Agent Toxins

Certain biological toxins are classified by the Federal Government as Select Agent due to their potential threat to public safety and health. The possession, use, or transfer of these biological toxins is highly regulated by the Federal Select Agent Program. Investigators using Select Agent Toxins are not required to register with the Select Agent Program if the amount does not exceed the permissible toxin amounts (see Appendix 1).

Investigators that possess a Select Agent Toxin less than or equal to the permissible amount must maintain an inventory of the amount of the Select Agent Toxin present in the laboratory. This inventory should document the number of vials, amount in each, amount remaining (if applicable) after each use, and how the toxin was inactivated when no longer needed for experiments. To meet this requirement, investigators should use the MSU Toxin Inventory Form.

The Federal Select Agent Program states that Investigators must show due diligence regarding any transfer of a Select Agent Toxin in order to prevent attempts by nefarious parties to stockpile toxins classified as a Select Agent Toxin. In accordance with 42 CFR 73.16, Investigators must document the recipient(s) of any Select Agent Toxin and provide evidence that the individual(s) has a legitimate purpose to possess toxins. Prior to any transfer of a Select Agent Toxin, Investigators must submit an Application for Request of an Excluded Select Agent Toxin form. This form must be approved by the IBC prior to shipping the toxin to the recipient.
Export Controlled Toxins

Certain biological toxins, including genetic elements encoding these toxins, are restricted for export by the U.S. Department of Commerce and are subject to Export Control regulations. To see if the toxin requires IBC approval, please consult the table in Appendix 1 or contact Justin Cook, Director of Research Compliance (jcook@montana.edu).

Table of Biological Toxins
The table indicates the LD50 for some acute biological toxins, the permissible amounts of Select Agent Toxins, and the toxins that are subject to Export Control regulations.

<table>
<thead>
<tr>
<th>Toxin</th>
<th>LD50 (µg/kg)</th>
<th>Requires IBC Approval</th>
<th>Select Agent</th>
<th>Export Compliance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abrin</td>
<td>0.7</td>
<td>Y</td>
<td>Y (100 mg)</td>
<td>Y</td>
</tr>
<tr>
<td>Aerolysin</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aflatoxins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-ungarotoxin</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Botulinum toxins</td>
<td>0.0004 to 0.0025</td>
<td>Y</td>
<td>Y (0.5 mg)</td>
<td>Y</td>
</tr>
<tr>
<td>Caeruleotoxin</td>
<td>53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cereolysin</td>
<td>40 to 80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholera toxin</td>
<td>250</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridium difficile</td>
<td>0.5 to 220</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>0.1 to 1500</td>
<td>Y</td>
<td></td>
<td>Y</td>
</tr>
<tr>
<td>Conotoxins</td>
<td>12 to 30</td>
<td>Y</td>
<td>a-conotoxins only, (100 mg)</td>
<td>Y</td>
</tr>
<tr>
<td>Crotoxin</td>
<td>12 to 30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diacetoxyscripenol toxin</td>
<td></td>
<td>Y</td>
<td>Y (1000 mg)</td>
<td>Y</td>
</tr>
<tr>
<td>Diphtheria toxin</td>
<td>0.1</td>
<td>Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HT-2 toxin</td>
<td>5 to 10</td>
<td></td>
<td></td>
<td>Y</td>
</tr>
<tr>
<td>Listeria listeriolysin or hemolysin</td>
<td>3 to 10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocidin</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microsystin (Cyanoginosin)</td>
<td></td>
<td></td>
<td></td>
<td>Y</td>
</tr>
<tr>
<td>Modeccin toxin</td>
<td>1 to 10</td>
<td></td>
<td></td>
<td>Y</td>
</tr>
<tr>
<td>Nematocyst toxins</td>
<td>33 to 70</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Notexin</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pertussis toxin</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumolysin</td>
<td>1.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosin exotoxin A</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ricin</td>
<td>2.7</td>
<td>Y</td>
<td>Y (100 mg)</td>
<td>Y</td>
</tr>
<tr>
<td>Saxitoxin</td>
<td>8</td>
<td>Y</td>
<td>Y (100 mg)</td>
<td>Y</td>
</tr>
<tr>
<td>Toxin</td>
<td>Concentration</td>
<td>Protection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------------</td>
<td>------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Shiga</em> Toxin</td>
<td>20</td>
<td>Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Shigella dysenteriae</em> neurotoxin</td>
<td>1.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcal aureus</em> toxins</td>
<td>2 to 25</td>
<td>Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Streptolysin</em> S</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Taipoxin</em></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Tetanus</em> toxin</td>
<td>0.001</td>
<td>Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T-2</em> toxin</td>
<td>5 to 10</td>
<td>Y (1000 mg)</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td><em>Tetrodotoxin</em></td>
<td>8</td>
<td>Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Verotoxin</em></td>
<td></td>
<td>Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Viscum Album lectin</em> 1 (Vixumin)</td>
<td>2.4 to 80</td>
<td>Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Volkensin</em> toxin</td>
<td>1.4</td>
<td>Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Yersinia pestis murine</em> toxin</td>
<td>10</td>
<td>Y</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix F - Record of Excluded Select Agent Toxin Use and Disposal

Record of Excluded Select Agent Toxin Use and Disposal

- Please use one form for each toxin purchase or acquisition.
- Keep this record for a minimum of three years after all of the toxin on this form has been destroyed.

Select Agent toxins are not required to register with the Federal Government if the amount under the control of a Principal Investigator does not exceed at any time the permissible toxin amounts, which are indicated below in parentheses. Principle Investigators that possess a Select Agent toxin equal to or under the permissible amount must maintain an inventory of the amount of the Select Agent toxin present in the laboratory at any given time. This inventory should document the number of vials containing toxin, amount in each, amount remaining (if applicable) after each use, and how the toxin was inactivated when no longer needed for experiments. You must also have a current IBC approval number and the appropriate training to possess any of the following Select Agent Toxins in any quantity:

- Abrin (100 mg)
- Botulinum neurotoxins (0.5 mg)
- Short, paralytic alpha conotoxins (100 mg)
- Diacetoxyscirpenol (DAS) (100 mg)
- Ricin (100 mg)
- Saxitoxin (100 mg)
- Staphylococcal Enterotoxins (subtypes A, B, C, D, and E) (5 mg)
- T-2 toxin (1000 mg)
- Tetrodoxin (100 mg)

<table>
<thead>
<tr>
<th>Building</th>
<th>Toxin Name:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room #</td>
<td>Total Amount Received:</td>
</tr>
<tr>
<td>Room Phone Number</td>
<td>Concentration:</td>
</tr>
<tr>
<td>Principal Investigator</td>
<td>Maximum Permissible Toxin Amount:</td>
</tr>
<tr>
<td>Principal Investigator Phone Number</td>
<td>LD50:</td>
</tr>
<tr>
<td>Lab Supervisor</td>
<td>Manufacturer/Source:</td>
</tr>
<tr>
<td>Lab Supervisor Phone Number</td>
<td>Catalog #:</td>
</tr>
<tr>
<td>IBC Protocol #</td>
<td>Ordered By (Name and Date):</td>
</tr>
<tr>
<td>IBC Approval Date</td>
<td>Received By:</td>
</tr>
<tr>
<td>Vial ID</td>
<td>Date and Time created</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------</td>
</tr>
</tbody>
</table>
### Appendix G - Summary of Requirements for Biosafety Levels

<table>
<thead>
<tr>
<th>Safety Guideline</th>
<th>BSL1</th>
<th>BSL2</th>
<th>BSL3</th>
<th>BSL4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory personnel must wash their hands after handling cultures, removing gloves, and before leaving the laboratory.</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Eating, drinking, and application of cosmetics is prohibited.</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Personnel must be familiar with basic biosafety procedures, including this manual.</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Personnel should wear goggles or face shields if the possibility of splashes and aerosols exists.</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Pipetting by mouth is prohibited.</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>All laboratory procedures should be performed to minimize aerosol generation.</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Work surfaces must be decontaminated at least daily, after each use for infrequent users, and after any spill of viable materials.</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Sharps must be placed in specially designed puncture- and leak-proof sharps containers and disposed of appropriately as medical waste.</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Laboratories must be kept neat; good housekeeping procedures must be in place and in regular use.</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>All medical waste is decontaminated before disposal by an approved decontamination method or disposed of as medical waste.</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Insect and rodent control programs are instituted.</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Laboratory contains a sink for handwashing.</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Laboratories are designed for ease of decontamination (e.g., no carpets, sealed surfaces, no unreachable areas, etc.).</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
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<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td><strong>Bench tops are impervious to water, moderate heat, and chemicals.</strong></td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td><strong>Laboratory furniture must be secured, and spaces between benches, cabinets, and equipment must be accessible for decontamination.</strong></td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td><strong>All laboratory windows must be fitted with fly screens.</strong></td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td><strong>Laboratory coats or gowns and gloves must be worn.</strong></td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Safety Guideline</td>
<td>BSL1</td>
<td>BSL2</td>
<td>BSL3</td>
<td>BSL4</td>
</tr>
<tr>
<td>---------------------------------------------------------------------------------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>Autoclaves are required for waste treatment prior to disposal as non-biohazardous waste.</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Autoclave quality control program is required for use specified above.</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Instructions for safety precautions are posted by the Principal Investigator.</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Animals not involved in the experiment are not permitted in laboratory.</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Biological safety cabinets are required and must be certified annually.</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Laboratory personnel require specific training in the handling of pathogenic materials.</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Safety centrifuge cups are required.</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Access to facility is limited or restricted during experiments.</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>The universal biohazard symbol must be posted on the access door to the laboratory.</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Immunization and/or serological testing for agents to be handled may be required.</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>All laboratory procedures must be performed in a properly certified biological safety cabinet.</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Laboratory requires controlled entry, unidirectional air flow, and other special design features.</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Windows must be closed and sealed.</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>No material or equipment can leave the laboratory unless it is autoclaved or decontaminated.</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Autoclaves must be located inside the laboratory.</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Access is through an airlock system.</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Y</td>
</tr>
</tbody>
</table>