Contents

Updates to Plan During Review: ................................................................. 5
Approvals ........................................................................................................ 6

Chapter 1: Biological Safety Program: Purpose, Scope, Responsibilities .............................................. 7
  Purpose .................................................................................................................. 7
  Scope ....................................................................................................................... 7
  Responsibilities .................................................................................................... 7

Chapter 2: IBC Approval of Research Projects ....................................................................................... 11
  Research and Activity Requiring Review and Approval from the IBC ................................................ 11
    IBC Protocol ........................................................................................................ 12

Chapter 3: Regulatory Guidelines ...................................................................................................... 13
  Federal, State, and Local Agency Regulations and Guidelines .................................................... 13
    Select Agent Rule ................................................................................................ 13
    Over Regulatory Requirements ............................................................................. 13

Chapter 4: Biohazardous Materials ...................................................................................................... 15
  Categories ............................................................................................................. 15
  Risk Groups .......................................................................................................... 15
  Tissue Culture/Cell Lines ....................................................................................... 16

Chapter 5: Routes of Transmission .................................................................................................... 17
  Skin and Mucous Membrane Contact ............................................................................. 17
  Ingestion .................................................................................................................. 17
  Percutaneous Inoculation ......................................................................................... 17
  Inhalation of Aerosols ............................................................................................. 17

Chapter 6: Biosafety Principles ......................................................................................................... 18
  Engineering Controls ............................................................................................. 18
  Personal Protective Equipment (PPE) ........................................................................... 18
  Standard Operating Procedures (SOPs) ................................................................... 18
  Administrative Controls .......................................................................................... 19

Chapter 7: Biosafety Levels .............................................................................................................. 20

Chapter 8: Laboratory Biosafety Practices ........................................................................................ 22
  Basic Laboratory Practices ....................................................................................... 22
  Laboratory Practice and Technique ........................................................................ 22
  Laboratory Housekeeping and Personal Hygiene ...................................................... 23
Updates to Plan During Review:

August 2020 Review:

General:
- Change rDNA to recombinant/synthetic nucleic acids
- General clarification points and edits

Purpose:
- “without limiting research” to “... minimal impact on research”
- “Chemical Safety Manual” to “Chemical Hazard Communications Plan”

Scope:
- “Materials that may harbor infectious organisms, such as human or primate tissues, fluids, cells, or cell cultures are considered biohazardous materials” to “Biohazardous materials include, but are not limited to, human or primate tissues, fluids, cells, or cell cultures; wastewater; plant pests and pathogens; plant products and soil.”

Responsibilities
- Added Biosafety Officer to first paragraph

Chapter 2
Research and Activities:
- Update list to be in line with IBC Manual

IBC Protocol
- Remove details of protocol lifecycle – Provide outline of lifecycle and notifications for review/renewal
  - Details captured in IBC Manual
- Update language to align with TOPAZ terminology

Chapter 3
USDA/APHIS/VS:
- Add information about plant/biofilm permitting

Chapter 4
Risk groups:
- Added RG4

Chapter 8
Security and Inventory or Biological Agents
- Add statement: “Each PI shall have an inventory of the biological materials stored in the laboratory.”

Chapter 13
Responding to a Spill
- Add “remove contaminated PPE and don new PPE prior to cleaning spill.

Appendix C
Updated Program text and removed form. Form now online.

Administrative Updates Since Last Review
- Updated links to BMBL (12/2020)
Approvals

Jovanka Voyich 9/9/2020
IBC Chair Signature Date
Jovanka Voyich-Kane

Ryan Bartlett 9/9/2020
Biosafety Officer Signature Date
Ryan Bartlett
Chapter 1: Biological Safety Program: Purpose, Scope, 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 MSU researchers, students, community and environment from biological hazards with minimal impact on 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 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 Hazard Communications Plan 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 pathogens/infectious agents and biohazardous materials. All faculty, staff, students, and visitors who work on MSU sponsored projects or at MSU facilities are included in the scope of this manual.

Biohazardous materials include, but are not limited to, human or primate tissues, fluids, cells, or cell cultures; wastewater; plant pests and pathogens; plant products and soil.

Responsibilities
Success of the Biosafety Program requires a team effort involving the Biosafety Officer, Institutional Biosafety Committee (IBC), Principal Investigators (PIs), laboratory workers, the Occupational Health Program, and Safety and Risk Management (SRM). PIs 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 biosafety standards in our research facilities.

Director of Research Compliance (IO)
The IO’s responsibility include:

- Oversight of biohazardous material usage in MSU research through a comprehensive biosafety program which outlines all aspects of dealing with such materials.
- Direct functional responsibility for the IBC and Biosafety Program.
- Effective communication between the IBC and other research related committees on campus.
- Appointment of committee members, in consultation with IBC chairperson.
**Institutional Biosafety Committee (IBC)**

The Institutional Biosafety Committee (IBC) is responsible for reviewing and approving research protocols involving recombinant/synthetic nucleic acids, and other biohazardous materials as outlined in the IBC Manual. The IBC is comprised of faculty representatives, from various academic disciplines, researchers, non-scientific members, students, and community representatives who are not affiliated with MSU. The IBC typically meets monthly to review research and other activities submitted to the IBC. 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:

- Oversight of MSU’s Biosafety Program, including development of new, and review of existing, policies and procedures designed to enhance the Biosafety Program.
- Review and approval of training programs.
- Coordination of biological safety requirements with other campus-wide committees (e.g., IACUC) and programs (e.g., Occupational Health Program).
- Review and approval of new research protocols and their modifications, involving rDNA and biohazardous material in accordance with guidelines established by the OSHA, USDA, CDC, NIH, and MSU policies.
- Define containment levels of biohazardous agents 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.
- Investigation of biosafety violations as well as significant accidents or illnesses involving biological agents.
- If appropriate, the IBC recommends disciplinary action to the appropriate MSU officials.
  - NIH, concerning rDNA exposures incidents
  - NIH’s Office of Biotechnology Activities, an IBC update

**Biosafety Officer (BSO)**

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, as well as federal, state, and local requirements. The program includes close cooperation and interaction with committees approving research protocols (IBC), procedures involving human subjects (Institutional Review Board (IRB)), Institutional Animal Care and Use Committee (IACUC), and Radiation Safety Committee (RSC). The BSO will also 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.

BSO’s responsibilities include:

- 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.
• Reviews all research proposals presented to the IBC.
• Helps ensure that biohazards, sharps, and glass wastes are properly treated, transported, and disposed of outside of laboratory facilities per applicable state and federal regulations.
• Maintains list of approved biosafety laboratories with inspection dates and results.
• Responsible for assisting the PIs in designing appropriate lab-specific biosafety manuals for all activities using potentially biohazardous materials

Principal Investigators (PIs)
The PI is primarily responsible for the people and activities in their laboratories and are responsible for implementing an appropriate biological safety program specific for their research projects. PIs should evaluate research operations, perform risk assessments, develop plans for all research activities accordingly, and ensure strict adherence to biological safety practices and techniques for all work involving potentially biohazardous materials.

The PI’s responsibilities include:

• Notifies the IBC regarding all research involving recombinant/synthetic nucleic acid molecules and/or biohazardous materials, submits an IBC protocol for approval, and adheres to all terms and conditions stipulated by the IBC therein.
• Ensures that all laboratory personnel are properly trained in the biosafety procedures and experimental techniques needed to ensure safety, as well as the MSU protocols for dealing with accidents and injuries.
• Informs the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested.
• Ensures that individuals working in the laboratory are experienced and proficient in handling biological agents.
• Makes available to all laboratory personnel the protocols that describe all biohazards and their associated precautions.
• Assumes responsibility for all safety practices, techniques, engineering controls and PPE that are required for any biohazards in his/her laboratory.
• Ensures that laboratory biohazards are effectively communicated to laboratory personnel and controls are in place to minimize risks associated with these hazards.
• Notifies the BSO 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 biohazardous 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 by a properly trained individual appointed by the PI.

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.

Occupational Health Program’s responsibilities include:

- Coordination with Bridger Occupational Health to provide medical evaluations and surveillance program for individuals.
- Filing and record keeping of Workman’s Compensation reports.
- Providing of annual respirator fit testing, and training.

Laboratory Workers
Laboratory workers are the most critical element in maintaining a safe working environment. Individuals must look out for their own safety and that of their co-workers. If individuals do not follow the MSU and laboratory-specific biosafety practices and procedures in the conduct of their laboratory duties, MSU cannot maintain a safe working environment.

Whoever works in the laboratory in a technical capacity is defined as a laboratory worker, whether the person is a PI, student, intern, visiting scholar, or volunteer.

Laboratory workers responsibilities include:

- Participate in and complete all required training to ensure that they are well-informed and up to date on biosafety responsibilities.
- Fully understand the biological agents and procedures used in the laboratory and the risks associated with exposure.
- Adhere to all laboratory practices, and protocols while complying with all applicable policies, procedures, and guidelines.
- Report to the supervisor or PI all problems, procedural discrepancies, spills, potential exposures, or accidental releases as soon as they occur.
- Complete any necessary medical surveillance.
Chapter 2: IBC Approval of Research Projects

Research and Activity Requiring Review and Approval from the IBC

The IBC reviews and approves many areas of biologically related activities, including research, teaching, and diagnostic projects.

The IBC defines biohazardous materials to include all infectious organisms that can cause disease in humans, animals, or plants, or have a significant negative environmental or agricultural impact. Any materials that are capable of harboring infectious organisms, such as human or primate tissues, fluids, cells, or cell cultures are considered biohazardous material.

Potentially biohazardous materials include but are not limited to the categories listed below. Projects involving any of these materials must be reviewed and approved by the IBC prior to initiation of the project.

- Recombinant/synthetic nucleic acid molecules.
- Genetically modified organisms (GMOs) including, but not limited to:
  - Animals, plants, invertebrates, and/or other organisms created by MSU employees or in/on MSU property.
  - Transgenic field trials, any genetically modified organisms to be 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 and pests: RG2/BSL2 or higher, human and animal pathogens, non-indigenous plant pathogens, as well as those plant and animal pests regulated by the USDA-APHIS.
- Select/Biological Agents and Toxins (CDC and USDA). 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 body 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 animals.
- Oncogenic viruses used in conjunction with animals.
- Any work that requires a USDA-Animal and Plant Health Inspection Service (APHIS) permit; in order to protect American agriculture and our natural resources, APHIS oversees and regulates the “import, transit and release of regulated animals, animal products, veterinary biologics, plants, plant products, pests, organisms, soil, and genetically engineered organisms.”

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 rDNA 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. The IBC protocol process is conducted in TOPAZ Elements. The PI applying for approval must submit an original protocol for review and approval.

A PI applying for approval of teaching activities involving potentially biohazardous material must contact the BSO. The BSO will assist the PI in developing a teaching protocol and determining the appropriate biosafety training for laboratory workers involved in the research activities. The PI is responsible for ensuring that all laboratory workers are trained prior to working with the infectious organisms and/or biohazardous material. The BSO will act as a resource to assist the PI in the IBC submission and approval process.

**Protocol Lifecycle**

*Requests for Amendments After IBC Approval*

All amendments to currently approved research, teaching 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 administrative modifications may include the addition of personnel and change of laboratory room (if change is to an equivalent and approved facility).

More significant IBC protocol modifications would include the addition of potentially biohazardous materials or procedures that would likely increase the safety risks to researchers. The PI must submit these modifications to the IBC for review. An IBC modification approval is valid until the end of the original approval period (3 years).

*Interim Review*

An Interim Review notice serves as a mechanism for the PI to provide an annual update of the research occurring on an IBC protocol. 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; report of any research-related problems/adverse events; and to provide a summary of the project over the last year.

If there are changes not covered in the approved protocol, the PI will need to submit a modification or in some cases may need to submit a new IBC protocol to cover the changes. As an essential part of the annual update process, all lab safety training requirements are verified as up to date, particularly for any new lab personnel.

*Renewal*

A Renewal Protocol is submitted every three years following initial approval. Renewal protocols are sent to full committee review. During renewal amending changes may be made to the protocol, if work outlined in the protocol has changed.
Chapter 3: Regulatory Guidelines

Federal, State, and Local Agency Regulations and Guidelines

The following is a summary of regulations and guidelines that cover the use of biological agents:

- Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH): Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th Edition, 2020. 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. The BMBL can be found using the following link: [https://www.cdc.gov/labs/BMBL.html](https://www.cdc.gov/labs/BMBL.html)

- National Institutes of Health (NIH): 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 (IBC) with the authority to approve proposed rDNA research using the NIH guidelines as the minimum standard. The NIH Guidelines can be found using the following link: [https://osp.od.nih.gov/biotechnology/nih-guidelines/](https://osp.od.nih.gov/biotechnology/nih-guidelines/)

- Occupational Safety and Health Administration (OSHA): Bloodborne Pathogen Standard. This regulation covers occupational exposure to human blood and other potentially infectious materials, including human tissues, body fluids, 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 or other potentially infectious materials must be offered immunization against hepatitis B virus (HBV) and receive annual training. Personnel who work with HIV or HBV in a research laboratory must receive additional training and demonstrate proficiency in working with human pathogens. The Bloodborne Pathogen Standard can be found using the following link: [https://www.osha.gov/laws-regs/regulations/standardnumber/1910/1910.1030](https://www.osha.gov/laws-regs/regulations/standardnumber/1910/1910.1030)

Select Agent Rule

US Department of Health and Human Services (HHS): 42 CFR Parts 42 and 43 Possession, Use, and Transfer of Select Agents and Toxins; 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.

Over Regulatory Requirements

U.S. Department of Transportation (USDOT) and the International Air Transportation Authority (IATA):

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 (USDA), Animal & Plant Health Inspection Service (APHIS), & Veterinary Services (VS):
These agencies regulate the import, export, movement and release of plants, plant pests, soil, genetically engineered organisms and biofilms.
These agencies also 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 approved several specific policies and procedures that are incorporated into this document as requirements or have been included as appendices.
Chapter 4: Biohazardous Materials

Categories
Biohazardous materials includes organisms that can cause disease in humans, animals, or plants; or have a significant negative environmental or agricultural impact. Any materials that are capable of harboring infectious organisms, such as human or primate tissues, fluids, cells, or cell cultures are also considered biohazardous material.

The following is a list of biohazardous materials.

- Infectious organisms (e.g., human, animal, plant, and other).
  - Viruses
  - Rickettsia
  - Chlamydia
  - Bacteria
  - Fungi
  - Parasites
  - Prions
- Toxins (bacterial, fungal, plant, etc.)
- Human and non-human primate cells. Including cell lines, tissue, blood and potentially infectious fluids.
- Work with animals/animal tissues 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.
- Cultured cells (all human or certain animal, including non-human primates) and the potentially infectious agents these cells may contain.
- Recombinant/synthetic nucleic acid molecules
- 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.

Prior to initiating any work with biohazardous material, the BSO must be contacted and an IBC protocol must be submitted and approved prior to initiating any work as outlined above in Chapter 2 and in the IBC Manual.

Risk Groups
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 (RG1)**
Agents not associated with disease in healthy adult humans.

**Risk Group 2 (RG2)**
Agents associated with human diseases that are rarely serious and for which preventive or therapeutic interventions are often available.

**Risk Group 3 (RG3)**
Agents associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk).

**Risk Group 4 (RG4)**
Agents associated with serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual and community risk).

**Tissue Culture/Cell Lines**

**Risk Group 1/Biosafety Level 1 (BSL1)**
The following are considered Risk Group 1 cell lines and can be handled using BSL1 containment:

- Cell lines of non-primate origin, which do not harbor a primate virus nor are they contaminated with an infectious organism.

**Risk Group 2/Biosafety Level 2 (BSL2)**
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 following are identified as Risk Group 2 and must be handled at BSL2 containment:

- Human and non-human primate cells including cell lines, tissue, blood and potentially infectious fluids.
- 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 unfixed human tissues and cells should be treated as infectious (the concept of “universal precautions”). All individuals who work in a laboratory containing these materials have the potential to be exposed to these materials. This requires these individuals must be included in the Bloodborne Pathogens program. These persons must be offered the hepatitis B vaccination and receive annual bloodborne pathogens training.
Chapter 5: Routes of Transmission

The risk of exposure to biological agents in a research environment depends on several 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 all result in the generation of infectious aerosols or droplets. Any direct contact between the infectious material and the subject’s skin, mucous membranes, or eyes may serve as a direct route of exposure.

Ingestion
Splashing of material into the mouth or indirect oral exposure through touching the mouth with contaminated hands can result in the ingestion of infectious material. Storage and/or consumption of food, drinks or eating utensils in the lab can also lead to ingestion of infectious material. Mouth pipetting presents an extremely high risk of ingestion and is prohibited at MSU.

Percutaneous Inoculation
Use of syringes and needles are considered the greatest risk of exposure through inoculation. Accidental inoculation can also occur by way of cuts and scratches incurred from contaminated syringes used for animal inoculations, or from the animals themselves in the form of bites or scratches.

Inhalation of Aerosols
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, general public, and the outside environment to potentially biohazardous materials. The four biosafety principles 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 controls designed to minimize exposure to biological agents.

Primary Engineering Controls

Primary engineering controls are designed to protect lab personnel and the immediate environment from exposure to infectious agents. They are the most effective at minimizing exposure when workers are trained in their proper use, and the equipment is regularly inspected and maintained. BSCs are the most important primary engineering control for protection of personnel and laboratory and may also provide protection for scientific samples and reagents.

Secondary Engineering Controls

Protecting the laboratory’s external environment from exposure to biohazardous materials is accomplished by a combination of biosafety principles and facility design. Facility airflow, access control, sinks for hand washing, self- closing doors, and heat-resistant and non-porous lab benches make up some of the most important 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. PPE should be complemented by wearing full-length trousers and closed-toe shoes (no sandals) in the laboratory setting. Proper clothing and PPE supplement the containment measures 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 (SOPs)

This document, the Biosafety in Microbiological and Biomedical Laboratories (BMBL), and the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules provide general requirements for working with biological agents. However, because these documents cover relatively general topics, individual laboratories are required to develop laboratory-specific SOPs which cover their own specific biosafety concerns and laboratory procedures.

For example, laboratory-specific SOPs should address safe manipulation of specific organisms, specific exposure control methods, 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 biohazardous materials. They include training, medical surveillance, vaccinations, and access control.
Chapter 7: Biosafety Levels

Four Biosafety Levels (BSL-1,2,3,4) 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 biohazardous material, 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” according to their relative pathogenicity, i.e., the ability to cause disease. Agents are categorized into four risk groups (RG).

In general, 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 in the table below.
<table>
<thead>
<tr>
<th>BSL</th>
<th>Agents</th>
<th>Practices</th>
<th>Primary Barriers and Safety Equipment</th>
<th>Facilities (Secondary Barriers)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Not known to consistently cause diseases in healthy adults</td>
<td>Standard microbiological practices</td>
<td>No primary barriers required. PPE: laboratory coats and gloves; eye, face protection, as needed</td>
<td>Laboratory bench and sink required</td>
</tr>
<tr>
<td>2</td>
<td>Agents associated with human disease</td>
<td>BSL-1 practice plus:&lt;br&gt;- Limited access  &lt;br&gt;- Biohazard warning signs  &lt;br&gt;- “Sharps” precautions  &lt;br&gt;- Biosafety manual defining any needed waste decontamination or medical surveillance policies</td>
<td>Primary barriers:&lt;br&gt;- BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials&lt;br&gt;- PPE: Laboratory coats, gloves, face and eye protection, as needed</td>
<td>BSL-1 plus:&lt;br&gt;- Autoclave available</td>
</tr>
<tr>
<td>3</td>
<td>Indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure</td>
<td>BSL-2 practice plus:&lt;br&gt;- Controlled access  &lt;br&gt;- Decontamination of all waste  &lt;br&gt;- Decontamination of laboratory clothing before laundering</td>
<td>Primary barriers:&lt;br&gt;- BSCs or other physical containment devices used for all open manipulations of agents&lt;br&gt;- PPE: Protective laboratory clothing, gloves, face, eye and respiratory protection, as needed</td>
<td>BSL-2 plus:&lt;br&gt;- Physical separation from access corridors&lt;br&gt;- Self-closing, double-door access&lt;br&gt;- Exhausted air not recirculated&lt;br&gt;- Negative airflow into laboratory&lt;br&gt;- Entry through airlock or anteroom&lt;br&gt;- Hand washing sink near laboratory exit</td>
</tr>
<tr>
<td>4</td>
<td>Dangerous/exotic agents which pose high individual risk of aerosol-transmitted laboratory infections that are frequently fatal, for which there are no vaccines or treatments&lt;br&gt;Agents with a close or identical antigenic relationship to an agent requiring BSL-4 until data are available to redesignate the level&lt;br&gt;Related agents with unknown risk of transmission</td>
<td>BSL-3 practices plus:&lt;br&gt;- Clothing change before entering  &lt;br&gt;- Shower on exit  &lt;br&gt;- All material decontaminated on exit from facility</td>
<td>Primary barriers:&lt;br&gt;- All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure suit</td>
<td>BSL-3 plus:&lt;br&gt;- Separate building or isolated zone&lt;br&gt;- Dedicated supply and exhaust, vacuum, and decontamination systems&lt;br&gt;- Other requirements outlined in the text</td>
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Chapter 8: Laboratory Biosafety Practices

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.

Techniques and practices are described in detail as “Standard Microbiological Practices” in the CDC/NIH’s Biosafety in Microbiological and Biomedical Laboratories and the NIH’s Guidelines for Research Involving Recombinant DNA Molecules, as well as in the National Research Council’s Biosafety in the Laboratory - Prudent Practices for the Handling and Disposal of Infectious Materials (National Academy Press, Washington, D.C., 1989). Many laboratory safety text and reference books also contain good information.

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.

Individuals working with infectious organisms or biohazardous 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 a lab-specific biosafety manual identifying specific hazards that
may be encountered, along with specific practices and procedures that will minimize risk(s) to lab personnel. All lab members should read and follow the required practices and procedures and be apprised of any special hazards. The PI or supervisor who is directing laboratory activities should be well-trained, experienced, and knowledgeable in the appropriate laboratory techniques, safety procedures, and hazards associated with the handling of infectious organisms and/or biohazardous material.

When standard laboratory practices are insufficient for the control of specific hazard(s) from an infectious organisms and/or biohazardous material or procedure, the PI should select additional safety measures to prevent exposure, and thereby ensure the safety of his/her lab personnel. These practices must also be supplemented by appropriate engineering controls, administrative controls, PPE, and SOPS.

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

**Laboratory Housekeeping and Personal Hygiene**

Personal safety is greatly enhanced by keeping the workspace neat, clean, and orderly. Injuries and exposures are more likely to occur in poorly maintained, disorderly areas. If workspace 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 must 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 must be avoided.
- Surfaces must 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 must be properly secured.

Personal hygiene, including proper handwashing techniques, is also a means of enhancing personal protection in the laboratory. Hand washing immediately after de-gloving ensures
that contamination of the hand by glove micro-puncture 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 a potential for exposure and may contribute to 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 activities clearly creates a heightened awareness of potential risks and adds another level of vigilance to activities involving reagents.

**Biological Hazard Information**

Laboratory workers should acquire extensive knowledge of the hazards associated with the infectious organisms or biohazardous materials in their labs. Furthermore, detailed hazard information, including Pathogen Safety Data Sheets, should be available to all laboratory workers.

**Storage and Labeling of Biological Agents**

Infectious organisms and biohazardous material must be stored using leak proof and sealed containers primary containers. Containers must be clearly labeled with the identity of the infectious organism and biohazardous material. At a minimum, secondary 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. 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 all containers of human blood or other potentially infectious material (OPIM), including contaminated waste, refrigerators, freezers, and other storage or transport vessels be labeled with the universal biohazard symbol.
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 Management 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-1 or BSL-2.
- Cages or animal rooms used for housing animals infected with BSL-1 or BSL-2 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 organisms or biohazardous material; 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. An MSDS 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 E and F. 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, several highly publicized incidents involving infectious organisms have increased both public concerns and regulatory oversight concerning the security of biological agents. Even though many of the infectious organisms 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/her laboratory implements sufficient security measures and procedures to prevent unauthorized access to biological agents.

Each PI shall have an inventory of the biological materials stored in the laboratory.

Prevention of Aerosols and Droplets

Handling of liquids or dry powders often generates 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; 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 BSO).
- The Magnehelic 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 airflow.
- The BSC should be left running whenever the cabinet is in use.
- Work surface should be wiped with 70% alcohol before and after 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 airflow before any procedures are begun.
- Any piece of equipment (i.e. centrifuge, blender) capable of creating air turbulence should be placed in the back one-third of the BSC. All other work should be stopped while this equipment is operating.
- Open flames are not allowed inside the BSC because they create airflow turbulence which compromises sterility. In addition, the heat buildup may damage the HEPA filters. Electric devices, such as loop sterilizers, are 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.”
- A 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 must not resume for 10 minutes.
- When work is complete, all materials must be removed from the BSC and all interior surfaces must be wiped with 70% ethanol, or other appropriate disinfectants.
- Gloves must be removed before exiting the BSC, after touching or handling contaminated materials.
- Laboratory coats 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 safe pipetting techniques will minimize the potential for exposure to hazardous materials:

- Never mouth pipette. Always use a pipetting aid.
- Do not prepare infectious organisms or biohazardous materials by bubbling expiratory air through a liquid with a pipette.
- Do not forcibly infectious organisms or biohazardous material out of a pipette.
- Never mix infectious organisms or biohazardous 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 above the receptacle. Whenever possible, allow the discharge to run down the container wall instead.
• 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 sharps container properly when it is, at most, three-fourths full.
• Pans or sharps containers for contaminated pipettes should be placed inside the BSC.
• Proper procedures for disposal of plastic pipettes are presented in Chapter 14.

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 cleaned regularly, maintained and used according to the manufacturer’s instructions. Users must be trained on proper operating instructions that include safety precautions of the centrifuge 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 also 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.
• Fill and open centrifuge tubes, rotors, and accessories in a BSC, if appropriate.
• 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 centrifuge 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.
• Work in a BSC 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 BSC during use to reduce the aerosol escape. High-speed centrifuges pose additional hazards.

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 all tissue remnants potentially infectious; carefully remove such accumulations from the cryostat during decontamination.

• Handle microtome knives with extreme care. Stainless steel mesh gloves should be worn when changing knife blades.

• Staining solutions used on potentially infected frozen sections should be treated as if they are contaminated.

Utilization of Inoculating Loops

Flaming inoculating loops can result in spatter and the release of aerosols and droplets. Use of an electric micro incinerator is the preferred, safer alternative when using a BSC.

Use of Absorbent Materials

Work surfaces should be covered with absorbent paper or “diaper” sheets to collect splashes and drips, thereby minimizing 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.

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 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.

• Blenders must be tested to ensure they are leak proof prior to use with any biohazardous material. Blenders can be tested with sterile saline or dye solution.

• The use of glass blender jars is not allowed because of the potential for breakage.

• 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 a minimum of five minutes to allow the aerosol to settle.

• Effective control of contamination can be achieved by placing the blender in a tray lined with absorbent pads, and inside a BSC.
• The device should be decontaminated after use.

Lyophilizers

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

• The vacuum pump exhaust must be filtered to remove any hazardous agents.
• After lyophilization is complete, all potentially exposed surfaces of the unit must be disinfected with the appropriate disinfectant.
• If the lyophilizer is equipped with a removable chamber, it should be closed off and moved to a BSC for unloading and decontamination.
• Vapor traps must be used whenever 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. Be aware of these hazards:

• 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.
• Observe all precautions listed above for blenders and lyophilizers.

Ampoules

Opening ampoules containing liquid or lyophilized culture material should be performed in a BSC to contain aerosols. 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.

Polypropylene cryovials are available in dust-free or pre-sterilized forms; each tube is fitted with a polyethylene cap and a silicone washer. 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 aerosolizing 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/biohazardous 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 unavailable.
• Continuous flame gas burners may not be used in a BSC. These burners can produce turbulence that disturbs the cabinet’s protective airflow patterns. Electric sterilizers and micro incinerators must be used when working in a BSC.
Chapter 9 – Engineering Controls

Primary Engineering Controls

Biological Safety Cabinets (BSC)

BSCs constitute one of the most critical pieces of safety equipment in Biosafety Level (BSL) Containment laboratories. Designed to contain aerosols generated from biological material via laminar air flow and high efficiency particulate air (HEPA) filtration, BSCs differ from chemical and laminar flow hoods (clean hoods) in that they always offer personnel projection. They also provide some protection from contamination of the material being handled within the work environment.

There are three types of BSCs (Class I, II, and III); each offers different levels of protection. Open-fronted Class I and Class II BSCs are partial containment devices that provide a primary barrier; significant levels of protection of personnel and environment are obtained when complemented by good laboratory technique. The gas-tight Class III BSC, or glove box, provides the highest level of protection to personnel, environment, and 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 protects personnel and 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 Types A and B may be found in the percent of air that is exhausted or recirculated, coupled with the way 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, environment, and 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 that 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, which prevents chemical fumes from exiting out of the hood. Instead, the air is exhausted to the outside, generally above the roof of the building. They do not provide HEPA filtered air at all, and are therefore unsuitable to protect personnel, product, or 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 user should observe the Magnehelic gauge and note its relative position. Magnehelic 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 does not have a Magnehelic gauge, users must understand the operation of the airflow monitor, controls, and alarm settings.

- Annually, the cabinet must be certified by an Asepsis Air Control technician (current service provider). The certification process is quick, 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, thereby enabling PIs to budget for the event.

- Annual BSC 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. Laboratory personnel should report lapsed BSC recertification to the Biosafety Officer immediately. The Biosafety Officer will inform the PI and lab workers not to use the BSC, post a “DO NOT USE” sign on it, and will arrange for Asepsis to get the BSC recertified as soon as possible. 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, 2020 will expire on June 30, 2021).

PIs are responsible for ensuring the proper maintenance and care of BSCs. Contact the BSO for more information.

Secondary Engineering Controls

Facility Design

The design of a laboratory facility is important in providing a barrier to protect both the personnel working within, as well as those outside of the laboratory. It should also protect the surrounding community and environment from accidental release of infectious agents in the lab. Facility design must be commensurate with the laboratory's function, particularly the BSL level required for use and/or storage of the biological agents therein.

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 exit, and a shower unit for personnel.

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 may 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; and separate buildings or modules for physical isolation of the laboratory itself.

*Note: Biosafety policy states that autoclaves used to sterilize biohazardous materials must be validated monthly; typically, a sporulation test is used. Validation records are essential. Biohazardous materials can also be disposed of in a red bag as medical waste (without autoclaving) and will be picked up by SRM. A nominal fee is charged for this service.
Chapter 10 - Personal Protective Equipment (PPE)

A Primary Barrier

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. Proper use of PPE significantly reduces the likelihood of infection; however, it is the least-desirable exposure control method because its failure will result in direct exposure to the agent.

PPE is most effective when used to supplement primary control methods such as BSCs, safety centrifuge cups, and other containment devices. Appropriate PPE and clothing also protect experiments 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 because contacts may trap infectious agents against the surface of the eye and prevent effective purging by eye washing.

Laboratory Clothing

Laboratory coats, smocks, scrub suits, and gowns are considered proper 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 (generally, autoclaving), should it become 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, as well as maintenance and service workers. All protective clothing should be discarded in the laboratory, disinfected, and/or laundered by the facility.
- Personnel must not launder laboratory clothing at home. If facilities/labs wish to have laboratory clothing laundered at a commercial laundry facility the laboratory clothing must be autoclaved prior to laundering.

Gloves

Gloves must be selected according to 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 SRM.

**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 practices, require that personnel be medically evaluated, specifically trained, and fit-tested prior to wearing respiratory protective equipment.

Contact SRM 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.

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**Chapter 11 - Training for Laboratory Personnel**
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/her daily laboratory safety practices.

At a minimum, all MSU personnel working with biological materials 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 agents and/or recombinant DNA (rDNA)
- 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 biosafety training as deemed appropriate by the IBC or the BSO.

The PI is responsible for ensuring that his/her employees receive proper training in the biohazards and controls specific to his/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 are also needed. 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 specific individual will depend on the hazards to which he/she is exposed. “Biosafety Training for Laboratory Workers” is a training program offered by the BSO and is designed for those working in laboratories.

This training is also offered through CITI and specific training requirements can be found on the MSU Biosafety webpage.

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.

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 BSO, who maintains the training records. 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 specific 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 to all laboratory personnel on a periodic basis. 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. This training is offered on demand through CITI, or periodically through the BSO.

**Laboratory-Specific Training**

Individual laboratories are required to develop training tailored for specific agents and/or 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. All laboratory personnel 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 be trained in the laboratory standard operating procedures (SOPs). Laboratory-specific training supplements general biosafety training.

**Training Records**

Each laboratory must maintain training records, which should include the names of lab personnel, and their completion dates for safety Training as well as lab- and/or agent-specific training provided by the PI or lab supervisor. All records must be updated and maintained in the Biosafety and Chemical Safety Logbooks 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., may also indicate the need for refresher training.

**Other Safety Training**

Personnel who utilize hazardous chemicals, radioisotopes, or x-ray-generating devices must attend additional safety training geared toward these specific hazards.

**Refresher Training**

All laboratory workers and certain categories of building occupants will be required complete refresher training. The scope and details of refresher training will be determined by the IBC and will range from annually (for those required by regulatory mandates, such as Bloodborne Pathogen Standard) to every three years.
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, 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 to swab an injection site on a person or animal, as well as 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 and is a vital component of microbiological safety practice. It not only serves to protect laboratory personnel (as well as any bystanders) from infection, but also prevents the release of infectious organisms to the outside environment. Decontamination of media, work surfaces, and equipment is also necessary to prevent contamination of experimentally cultured organisms.

Infectious waste materials such as liquid and solid will be handled, treated and disposed of according to hazardous waste policies and procedures. Liquid wastes such as bacterial or viral culture media from BSL-2 labs will be treated with appropriate disinfectant prior to sink disposal. Solid waste from BSL-2 laboratories will be segregated and placed in biohazard containers lined with biohazardous waste bags and disposed as biological waste.

- Autoclaving is the preferred method for treating biological waste.
- A disinfectant should be chosen that is appropriate for the organism in use.
- All liquid biological cultures should be inactivated 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 with a chamber temperature of at least 121° C/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. Biohazard bags should be loosely closed to allow trapped air to escape.
- Sterilization requires that materials come in direct contact with the steam and heat. Indicators of proper autoclave operation (e.g., autoclave tape or autoclave-sensitive labels) must be used with each load to visually confirm successful processing.
- Use of autoclave tape alone is not an adequate monitor of autoclave performance. As
mentioned previously, Biosafety policy requires the use of a well-documented, monthly sporulation test to confirm sterilization.

- **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 to kill all pathogenic agents present. Each specific disinfectant requires its own specific 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 121° C for at least 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/320°-356° F 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, BSCs, 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 chemical disinfectants.

- Inhalation and skin contact should be minimized, and eye contact avoided; PPE is essential.
- Appropriate gloves and safety eyewear should always be worn when handling these chemicals.
- Pertinent information for common chemical disinfectants is summarized in table below.

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>Use Parameters:</th>
<th>EFFECTIVE vs.: a</th>
<th>Important Characteristics</th>
<th>Potential Application</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alcohol</strong> (ethyl, isopropyl)</td>
<td>70-85% 10-30 min.</td>
<td>+ + + ±</td>
<td>TOXIC: Eye irritant; flammable; inactivated by organic matter.</td>
<td>Surfaces: work and equipment</td>
</tr>
<tr>
<td>Chlorine Compounds (Cpds)</td>
<td>0.05-0.5% (bleach is 0.5%) 10-30 min.</td>
<td>+ + + + ±</td>
<td>May leave residue; corrosive; skin, eye &amp; respiratory irritant; inactivated by organic matter; Unstable; make up weekly.</td>
<td>Spills, equipment surfaces Instruments; glassware; water baths.</td>
</tr>
<tr>
<td>Quaternary Ammonium Cpds</td>
<td>0.1-2% 10-30 min.</td>
<td>+ +</td>
<td>TOXIC; inactivated by organic matter.</td>
<td>Surfaces (work and equipment); BSCs; floor maintenance; glassware; instruments</td>
</tr>
<tr>
<td>Phenolic Cpds</td>
<td>0.2-3% 10-30 min.</td>
<td>+ + + ±</td>
<td>TOXIC; Eye &amp; respiratory irritant; inactivated by organic matter.</td>
<td>Surfaces (work and equipment); BSCs, floors, spills, glassware, instruments, water baths.</td>
</tr>
<tr>
<td>Iodophor Cpds</td>
<td>0.47% 10-30 min.</td>
<td>+ + + ±</td>
<td>Leaves residue; corrosive; TOXIC; skin &amp; eye irritant; inactivated by organic matter.</td>
<td>Surfaces (work &amp; equipment); BSCs, glassware, water baths</td>
</tr>
<tr>
<td>Formaldehyde b</td>
<td>4-8%</td>
<td>+ + + + ±</td>
<td>Leaves residue; skin &amp; eye irritant.</td>
<td>Less effective than others</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 & status as a carcinogen, formaldehyde should not be used without good local exhaust ventilation.

From Laboratory Safety: Principles and Practices, second edition, Diane O. Fleming, John H. Richardson, Jerry J. Tulis, Donald Vesley, eds;
Amer Society for Microbiology (1995).
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.

Preparing Ahead of Time 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., BSC, 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. PIs or laboratory directors should prepare their laboratory for typical spill scenarios expected in the lab. Laboratory workers should be informed of the hazards of the biological agents being used, 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, 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 SRM 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 BSO at (406) 994-6733.

**Biohazardous Spills Inside Biosafety Cabinets (BSCs)**

- Wear a lab 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 BSC

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.
• 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 and contain 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 for autoclaving (biohazard waste bag, sharps container, or autoclavable pan with lid for reusable items).
• 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.

Disinfect all items in the spill area.

• Allow a minimum of 30 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 for autoclaving (biohazard waste bag, sharps container, or autoclavable pan with lid for reusable items).
• 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 aerosols to settle (usually a minimum of 30 minutes) before re-entering the area.
• Wear a lab coat (disposable recommended), safety glasses, and gloves during cleanup.
• 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 BSO (406)-994-6733

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.
- Wash any wounds immediately with soap and water.
- Await assistance: Unless laboratory personnel are trained and properly supplied with PPE, DO NOT attempt to clean up the spill. Personnel should immediately call the Biosafety Office at (406)-994-6733
- 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.
- Remove contaminated PPE and don new PPE prior to cleaning spill.
- 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 SRM personnel and await arrival of the emergency provider.
- Clean up: Clean up should take place ONLY if laboratory personnel are trained, properly supplied with PPE, 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, 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 waste, 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 all 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 (i.e. “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 (contaminated or not), 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 SRM.

**Uncontaminated Laboratory Glassware and Broken Glass**

Collect uncontaminated laboratory glassware and broken glass in designated rigid containers (separate from other waste) that will prevent cuts and punctures to personnel. Containers should be labeled “broken glass.” The rigid containers with broken glass (often a cardboard box) are eventually taped shut and disposed of with the 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, loosely tied shut, labeled, and finally 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 SRM.

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

- Infectious animal carcasses are placed in a red biohazard bag secured with a plastic tie and incinerated through the Animal Resources Center.
- 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, which govern materials that are transported through the “public domain,” (i.e. 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.
- Copies of this information should be place both on the outside and inside of shipping container.
- 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 US 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 prepare 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, rickettsia, 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, possesses the capability to cause permanent disability or life-threatening 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 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 packaged:

- 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 making contact.
- 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 A label]

**Category B:**

![Category B label]
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 requires the following:

- 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 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.

<table>
<thead>
<tr>
<th>S</th>
<th>INSTRUCTIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Shipper’s:</td>
</tr>
<tr>
<td></td>
<td>♦ Name</td>
</tr>
<tr>
<td></td>
<td>♦ Address</td>
</tr>
<tr>
<td></td>
<td>♦ Phone number</td>
</tr>
<tr>
<td>2</td>
<td>Receiver’s:</td>
</tr>
<tr>
<td></td>
<td>♦ Name</td>
</tr>
<tr>
<td></td>
<td>♦ Address</td>
</tr>
<tr>
<td></td>
<td>♦ Phone number</td>
</tr>
<tr>
<td>3</td>
<td><strong>Line out the item that does not apply.</strong> 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.</td>
</tr>
<tr>
<td>4</td>
<td><strong>Line out the item that does not apply.</strong></td>
</tr>
<tr>
<td>5</td>
<td>♦ Proper Shipping Name (infectious substance, affecting humans or infectious substance, affecting animals)</td>
</tr>
<tr>
<td></td>
<td>♦ Identify the specimen by name in parenthesis</td>
</tr>
<tr>
<td></td>
<td><strong>ex. Infectious substance, affecting humans (rabies virus)</strong></td>
</tr>
<tr>
<td>6</td>
<td><strong>Class or Division</strong> * Always 6.2**</td>
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<tr>
<td></td>
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<tr>
<td>---</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>7</td>
<td><strong>UN Code</strong> * UN 2814 or UN 2900 (UN 3373 does not require shippers dec.)</td>
</tr>
<tr>
<td>8</td>
<td><strong>Packaging Group</strong> * There is no packaging group for biological agents.</td>
</tr>
<tr>
<td>9</td>
<td>♦ Identify by stating the number of containers by the quantity in each container. (e.g., 5 X 10ml)</td>
</tr>
<tr>
<td></td>
<td>♦ Identify type of outer container for the shipment</td>
</tr>
<tr>
<td>10</td>
<td><strong>Packaging Instructions</strong> * 602 or 650 (also 904 if dry ice included)</td>
</tr>
<tr>
<td>11</td>
<td>♦ 24-hour emergency contact number for the shipper (PI, Lab Supervisor),</td>
</tr>
<tr>
<td></td>
<td>♦ 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.”</td>
</tr>
<tr>
<td>12</td>
<td><strong>Name and Signature of the shipper.</strong></td>
</tr>
</tbody>
</table>
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, rickettsia, 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 to avoid exposing the package contents to package handlers, transporters, and the 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

Categories of Laboratory-ventilated containment equipment.

Chemical Fume Hoods (CFH), Laminar Flow Clean Benches (LFCB), and Biological Safety Cabinets (BSC)

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

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 damage. 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):**

- Inflow test
- Down-flow test
- Smoke pattern test
- HEPA filter leakage
- Cabinet leakage (when BSC is newly installed, relocated, or maintenance has been performed that involved removal of access)
**Additional tests (worker comfort and safety):**

- Noise
- Vibration
- Lighting
- Electrical leakage, polarity, and ground circuit resistance

<table>
<thead>
<tr>
<th>New NSF Classification, Adopted 2002</th>
<th>Previous NSF Classification</th>
<th>General Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 Class II, Type A</td>
<td>70% air recirculated; 30% exhausted from a common plenum to the room; 75FPM intake; may have biologically contaminated positive pressure plenum</td>
<td></td>
</tr>
<tr>
<td>A2 Class II, Type A/B3</td>
<td>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</td>
<td></td>
</tr>
<tr>
<td>A2 Class II, Type B3</td>
<td>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</td>
<td></td>
</tr>
<tr>
<td>B1 Class II, Type B1</td>
<td>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</td>
<td></td>
</tr>
</tbody>
</table>
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 validated autoclave, these materials are considered non-biohazardous. Materials that contain hazardous chemicals, including bleach, must not be autoclaved.

This assurance program applies to all autoclaves used to sterilize waste from Biosafety Level 1 and 2 laboratories.

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

Biohazardous waste must be treated in an autoclave for a minimum of 60 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.). Sterilization by autoclaving is accomplished through exposure and penetration of the contaminated material by 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. Liquid waste may also be autoclaved instead of adding chemical disinfectants and disposed in the sink. Autoclaved waste must include a steam sterilization indicator strip and/or autoclave tape. The operating autoclave temperature and time must be verified for each run. Records of the temperature and time must be maintained for at least one year.

On a monthly basis (at a minimum), confirm that adequate sterilization occurs through the use of Biological Indicators (BIs). Place BIs in the center of autoclave bags (dry loads) or in the liquid (liquid loads) of an autoclave load. There are specific BIs for both dry and liquid loads. Record monthly BI results in the Autoclave Quality Assurance Form and submit for records.

Monthly Spore Testing Procedure
Place BIs in the center of autoclave bags (dry loads) or in a container with water (liquid load) of an autoclave load. You may tie string to the indicators and tape the string to the outside of the bag/container for easy retrieval.

Note: The container of water for liquid loads should represent the type of container used and the largest volume of liquid waste to be autoclaved.

Run the autoclave load under normal operating procedures.

Incubate the autoclaved BIs and a non-autoclaved control BI according to the manufacturer’s instructions (normally 55°-60° C for 24 to 48 hours).

If a color change occurs in an autoclaved BI, the sterilization process was unsuccessful. Discontinue use of the autoclave until it is repaired and passes retesting. Tag the autoclave as “Not Approved for Biohazardous Waste” until the autoclave passes retesting.
Biological indicator sources:
https://sterilizermonitoring.mesalabs.com/mtc/Products/List?cid=2
https://www.fishersci.com/shop/products/3m-attest-biological-indicator/nc0413414
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 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. Plastic ware 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 instead 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, workspaces 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 embryonated 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 to 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 be reported to ORC/SRM 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 can support 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 \( \leq 100 \text{ ng/kg} \) 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 BSO. Investigators working with a biological toxin that require IBC approval must submit an IBC Protocol.

Work involving recombinant or synthetic DNAs that encode the active subunit(s) of a biological toxin with an LD$_{50}$ of \( \leq 100 \mu\text{g/kg} \) 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
nfarious 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 Kirk Lubick, Director of Research Compliance (kirk.lubick@montana.edu).

**Table of Biological Toxins:** LD$_{50}$ for some acute biological toxins, permissible amounts of Select Agent Toxins, and toxins that are subject to Export Control regulations.

<table>
<thead>
<tr>
<th>Toxin</th>
<th>LD$_{50}$ (µg/kg)</th>
<th>IBC Approval</th>
<th>Select Agent</th>
<th>Export Compliance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abrin</td>
<td>0.7</td>
<td>YES</td>
<td>Y (100 mg)</td>
<td>YES</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-bungarotoxin</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Botulimum toxins</td>
<td>0.0004 to 0.0025</td>
<td>YES</td>
<td>Y (0.5 mg)</td>
<td>YES</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>YES</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>YES</td>
<td></td>
<td>YES</td>
</tr>
<tr>
<td>Conotoxins</td>
<td>12 to 30</td>
<td>YES</td>
<td>α- only (100 mg)</td>
<td>YES</td>
</tr>
<tr>
<td>Crotoxin</td>
<td>12 to 30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dia cetoxys cripenol toxin</td>
<td></td>
<td>YES</td>
<td>Y (1000 mg)</td>
<td>YES</td>
</tr>
<tr>
<td>Diphtheria toxin</td>
<td>0.1</td>
<td>YES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HT-2 toxi n</td>
<td>5 to 10</td>
<td></td>
<td></td>
<td>YES</td>
</tr>
<tr>
<td>Listeria listeriol ys in or hemol ys in</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>Mi cros ys tin (Cya noginosin)</td>
<td></td>
<td></td>
<td></td>
<td>YES</td>
</tr>
<tr>
<td>Modecci n toxin</td>
<td>1 to 10</td>
<td></td>
<td></td>
<td>YES</td>
</tr>
<tr>
<td>Nema tocs t toxi ns</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>Toxin</td>
<td>IC50</td>
<td>YES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>------</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 aeruginosa exotoxin A</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rcin</td>
<td>2.7</td>
<td>YES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shiga Toxin</td>
<td>20</td>
<td>YES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shigella dysenteriae neurotoxin</td>
<td>1.3</td>
<td>YES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcal aureus toxins</td>
<td>2 to 25</td>
<td>YES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptolysin S</td>
<td>25</td>
<td>YES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetaxin</td>
<td>2</td>
<td>YES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-2 toxin</td>
<td>0.001</td>
<td>YES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetrodotoxin</td>
<td>5 to 10</td>
<td>YES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verotoxin</td>
<td>8</td>
<td>YES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viscum Album lectin 1 (Vixumin)</td>
<td>2.4 to 80</td>
<td>YES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volkensin toxin</td>
<td>1.4</td>
<td>YES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yersinia pestis murine toxin</td>
<td>10</td>
<td>YES</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix F - Record of Excluded Select Agent Toxin Use & 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:

Recently updated allowable amounts (2/2017).

[https://www.selectagents.gov/PermissibleToxinAmounts.html](https://www.selectagents.gov/PermissibleToxinAmounts.html)

<table>
<thead>
<tr>
<th>HHS Toxins [§73.3(d)(7)]</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abrin</td>
<td>1000 mg</td>
</tr>
<tr>
<td>Botulinum neurotoxins</td>
<td>1 mg</td>
</tr>
<tr>
<td>Short, paralytic alpha conotoxins</td>
<td>100 mg</td>
</tr>
<tr>
<td>Diacetoxyscirpenol (DAS)</td>
<td>10,000 mg</td>
</tr>
<tr>
<td>Ricin</td>
<td>1000 mg</td>
</tr>
<tr>
<td>Saxitoxin</td>
<td>500 mg</td>
</tr>
<tr>
<td>Staphylococcal Enterotoxins (Subtypes A, B, C, D, and E)</td>
<td>100 mg</td>
</tr>
<tr>
<td>--------------------------------------------------------</td>
<td>--------</td>
</tr>
<tr>
<td>T-2 toxin</td>
<td>10,000 mg</td>
</tr>
<tr>
<td>Tetrodotoxin</td>
<td>500 mg</td>
</tr>
<tr>
<td>Building</td>
<td>Toxin Name:</td>
</tr>
<tr>
<td>----------</td>
<td>-------------</td>
</tr>
<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>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></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>BSL-1</th>
<th>BSL-2</th>
<th>BSL-3</th>
<th>BSL-4</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>Bench tops are impervious to water, moderate heat, and chemicals.</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Laboratory furniture must be secured, and spaces between benches, cabinets, and equipment must be accessible for decontamination.</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>All laboratory windows must be fitted with fly screens.</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
</tbody>
</table>
Laboratory coats or gowns and gloves must be worn. | Y | Y | Y | Y
<table>
<thead>
<tr>
<th>Safety Guideline</th>
<th>BSL-1</th>
<th>BSL-2</th>
<th>BSL-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoclaves are required for waste treatment prior to disposal as non-biohazardous waste.</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>
</tr>
<tr>
<td>Instructions for safety precautions are posted by the Principal Investigator.</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>
</tr>
<tr>
<td>Biological safety cabinets are required and must be certified annually.</td>
<td>N</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>
</tr>
<tr>
<td>Safety centrifuge cups are required.</td>
<td>N</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>
</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>
</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>
</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>
</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>
</tr>
<tr>
<td>Windows must be closed and sealed.</td>
<td>N</td>
<td>N</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>
</tr>
<tr>
<td>Autoclaves must be located inside the laboratory.</td>
<td>N</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>Access is through an airlock system.</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>
Appendix H - Biosafety Practices for Teaching Laboratories- ASU

Used with Permission from the Arizona State University Institutional Biosafety Committee

Biosafety Practices for Teaching Laboratories

Introduction

In 2012, the American Society for Microbiology (ASM) published a document titled, Guidelines for Teaching Laboratories. These guidelines recommend procedures for working at Biological Safety Level 1 (BSL-1) and Biological Safety Level 2 (BSL-2) in teaching laboratories. The ASM publication was influenced by the lack of safety guidelines for microbiology teaching laboratories and a multistate outbreak of Salmonella typhimurium originating in teaching and clinical laboratories in 2011. A major culprit identified during an investigation of the outbreak and a similar one in 2014 was a lack of biosafety training and awareness for staff and students.

Arizona State University (ASU) has many teaching labs at the introductory, intermediate, and advanced undergraduate levels, as well as graduate levels. Environmental Health and Safety (EH&S) has compiled these Biosafety Practices for Teaching Laboratories with input from the Institutional Biosafety Committee (IBC), instructors, and other key stakeholders, to ensure that teaching laboratories are safe for students, staff, visitors, and inspectors. The primary purpose of this document is to prevent pathogen exposure to personnel and the community, and to prevent releases to the environment.

Specifically, this document contains biosafety requirements for teaching laboratories operating at BSL-1 and BSL-2 and is intended to supplement the detailed resources provided in the MSU Biosafety Manual. The IBC must approve all teaching laboratories using any materials requiring BSL-2 containment. It is important to note that not all teaching laboratories are designed or equipped to safely operate at BSL-2. This is one reason why it is so important to have an IBC protocol submitted before any BSL-2 work is performed in a teaching laboratory. Please contact the Office of Research Compliance or the Biosafety Officer (994-3779) with any questions or clarifications regarding assigned biosafety levels.

Subculturing Unknown Samples

Students are permitted to culture organisms from soil, water, food materials, and the air in the teaching laboratory. If the samples will be used to only count and understand the types of organisms in a particular environment, and no subculturing is performed, then IBC approval is not required. Sub culturing from an initial culture plate requires IBC review and approval, especially if differential media is used in the experiment. In addition, if the laboratory will include sub culturing and isolation from environments such as water fountains, door handles, wastewater treatment outfalls, or other areas that could harbor pathogens, review and approval by the IBC must be obtained. Samples must never be cultured from the students’ bodies without approval from the IBC, and possibly the Institutional Review Board (IRB), as there may be the potential to grow microbes that require BSL-2, or even BSL3 containment.

Note: Instructors are encouraged to create “unknown” samples for students from a mixture
of known microorganisms (selected by the instructor), or from a culture where the instructor knows the contents, instead of using samples from the environment.

Minors Working in Biological Laboratories
All minors working in research and teaching laboratories must have their research projects approved by the ORC, IBC, SRM, and all procedures must adhere to the University policy. Minors in these laboratories are permitted to work with well-established BSL-1 materials only, unless approved by the ORC, IBC and EH&S.

Biological Safety Level One (BSL-1) Requirements
All information regarding biosafety and biosecurity should be included in the course syllabus. This will help ensure easy access to this important information at any time during the course.

**BSL-1 Laboratory Facility Requirements**
BSL-1 includes microorganisms that are not known to cause human disease, and that may be handled safely on bench tops. The use of BSL-1 containment is the most appropriate for most teaching laboratories. However, some facilities may not meet these requirements due to the original design requirements for the laboratory space. Any facility renovation or new construction must include the following requirements:
- Non-porous flooring, bench tops, chairs, and stools
- Sink for hand-washing
- Eyewash station
- Lockable door to the laboratory
- Proper pest control practices
- Recommended: Separate storage area for personal belongings
- Recommended: Access to a working and validated autoclave

**BSL-1 Stock Culture Requirements**
Always provide students with an exceptional learning experience by ensuring that they are working with known and well-documented stocks.
- Stock cultures must be from approved and reputable sources.
- Sub culturing microbes isolated from the environment, clinical samples, or other unknown locations is discouraged as BSL-2 (or higher) microbes may be isolated.
- Sub culturing from the environment must be reviewed and approved by the IBC.
- When possible, only well-characterized microbes should be used (e.g., identified with an ATCC number) and examples are provided in Table 1.
- The laboratory instructor must maintain safety documentation for all stock organisms, sources, and procedures for handling stock cultures.
- Fresh stock cultures of microorganisms must be obtained on a regular basis (at least annually) to be certain of the source culture, to minimize spontaneous mutations, and to reduce contamination.

**Table 1. Recommended BSL-1 Agents for Teaching Laboratories**
<table>
<thead>
<tr>
<th>Microorganism</th>
<th>ATCC Number</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alcaligenes faecalis</em></td>
<td>1 8750</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>1 16888</td>
</tr>
<tr>
<td><em>Bacillus globigii</em></td>
<td>1 69510</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>1 23857</td>
</tr>
<tr>
<td><em>Bacillus megaterium</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Citrobacter freundii</em></td>
<td>1 8090</td>
</tr>
<tr>
<td><em>Clostridium sporogenes</em></td>
<td>1 3584</td>
</tr>
<tr>
<td><em>Enterobacter aerogenes</em></td>
<td>1 13048</td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Enterococcus casseliflavus</em></td>
<td>1 700327</td>
</tr>
<tr>
<td><em>Enterococcus durans</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Escherichia coli B</em></td>
<td>1 11303</td>
</tr>
<tr>
<td><em>Escherichia coli K12</em></td>
<td>1 10798</td>
</tr>
<tr>
<td><em>Geobacillus stearothermophilus</em></td>
<td>1 12980</td>
</tr>
<tr>
<td><em>Halobacterium salinarum</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Klebsiella oxytoxa</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Lactobacillus acidophilus</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Micrococcus luteus</em></td>
<td>1 4698</td>
</tr>
<tr>
<td><em>Neurospora crassa</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Penicillium chrysogenum</em></td>
<td>1 10106</td>
</tr>
<tr>
<td><em>Providencia alcalifaciens</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Pseudomonas fluorescens</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Rhanella aquatilis</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Rhizopus stolonifer</em></td>
<td>1 14037</td>
</tr>
<tr>
<td><em>Rhodococcus rhodocci</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em></td>
<td>1 9763</td>
</tr>
<tr>
<td><em>Sarcinia aurantiaca</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Serratia liquefacens</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>1 13880</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>1 14990</td>
</tr>
<tr>
<td><em>Staphylococcus saprophyticus</em></td>
<td>1 15305</td>
</tr>
</tbody>
</table>

Examples of BSL-1 Experiments (Using Non-Infectious Agents)

ASU instructors have many years of experience in determining which experiments are appropriate for novice students. The following experiments are those deemed most appropriate for introductory microbiology teaching laboratories:

- Anaerobic growth
- Bacterial enumeration
- Bacterial transformation
- Capsule stain
- Carbohydrate fermentation
- Casein hydrolase
- Catalase and oxidase test
- Endospore stain
- Eosin methylene blue plate
- Flagella stain
- Gel electrophoresis
- Gelatin hydrolysis
- Gram stain
- Hanging drop
- Indole methyl red Vogues-Proskauer and Citrate (IMViC)
• Kirby-Bauer
• Litmus milk
• Luria broth
• MacConkey Agar
• Mannitol, nitrate reduction
• 4-Methylumbelliferyl-β-D-glucuronide Escherichia coli broth medium (E. coli MUG)
• Plasmid DNA isolation
• Pour and quadrant streak plate
• Restriction endonuclease digestion
• Spread
• Starch hydrolysis
• Transformation assay
• Triple sugar iron
• Urease
• Use of lambda bacteriophage

BSL-1 Personal Protective Equipment Requirements

• Safety goggles or safety glasses (with side shields) must be worn when handling stocks and liquid cultures, spreading plates, or when performing procedures that may create a splash. If glasses are shared among students, they must be sanitized with an appropriate disinfectant after use.
• Note that the disinfectant to be used and disinfection time must be documented in the syllabus.
• Long pants or long skirts (ankle length) or other clothing to cover exposed skin must be worn.
• Closed toe and closed heel shoes covering the top of the foot must be worn.
• Gloves must be worn when the student has fresh cuts or abrasions on the hands, when staining microbes, and when handling hazardous chemicals.
• Gloves must be worn when handling cultures.
• Hands must be washed immediately after handling microbial cultures and anytime accidental contact occurs with the skin.
• Hand cleansing must be performed with soap and water, and if none is available with ethanol based hand sanitizer. Soap and water must be used as soon as possible if hand sanitizer is used.
• Recommended: Laboratory coats should be worn when handling cultures.

BSL-1 Work Practices

• Hands must be washed after entering and before leaving the laboratory.
• Long hair must be tied back.
• Long, dangling jewelry is not permitted in the laboratory.
• Benches must be disinfected upon entering the laboratory and at the end of the laboratory session. Any materials that are spilled must be immediately cleaned-up. Disinfectants used must be effective against microbes used in the laboratory. EH&S Biosafety and Biosecurity may be consulted for disinfectant recommendations.
• Food, water bottles, gum, and drinks of any kind are prohibited in the laboratory.
• Students should not touch their faces, apply cosmetics, adjust contact lenses, bite nails, or chew on pens/pencils in the laboratory.
• All personal items must be stowed in a clean area while in the laboratory. The use of cell phones, tablets, and other personal electronic devices is discouraged.
• Mouth pipetting is prohibited.
• All containers must be labeled clearly with the contents.
• The laboratory door must remain closed at all times when the laboratory is in session. The laboratory instructor must approve all persons entering the laboratory.
• Sharps usage must be minimized. Needles and scalpels are to be used according to ASU guidelines. All sharps (includes coverslips, slides and Pasteur pipets) must be disposed in a sharps container.
• Waste materials from the laboratory must be disposed properly.
• Test tube racks or other secondary containers must be used to move cultures in the lab.
• Stocks and other cultures must be stored in a leak-proof container when work is complete.
• Broken glass must be handled using a dustpan and broom or forceps and tongs. Students and laboratory personnel must not pick up broken glass with their hands. If contaminated, broom and other tools used to pick up broken glass must be disposed or sterilized.
• All spills or injuries must be immediately reported to the laboratory instructor.
• Teach, practice, and enforce the proper wearing and use of personal protective equipment.
• Advise immune-compromised students and those living with or caring for an immune compromised person to consult physicians to determine the appropriate level of laboratory participation. Students should not be asked to reveal if they are immunocompromised. A general announcement should be made that students with a reduced immune status should consult with University Health Services. A note from University Health Services is sufficient to excuse a student from laboratory work.
• Recommended: Supply pens and pencils for students and keep separate from personal items.
• Recommended: Keep note taking and discussions separate from work with lab materials.
• Recommended: Use micro-incinerators rather than Bunsen burners.

BSL-1 Training Practices
• Faculty and teaching assistants must complete all required safety trainings prior to beginning the first day of class and annually thereafter.
• Instructors and/or teaching assistants must review basic biosafety and microbiological practices with students on the first day of the laboratory. The requirements listed in this document should be included in this training session. The training session must be documented with a sign-in sheet maintained by the instructor. This may be performed using an online system such as Blackboard.
• Students and instructors are required to handle microorganisms safely and in conjunction with requirements outlined in the MSU biosafety manual.
• Students must be informed of safety precautions applicable to each exercise before the procedure is performed.
BSL-1 Documentation

- Safety Data Sheets (SDS) must be available for all chemicals in the laboratory.
- Students must sign safety agreements indicating that they have been informed about the safety requirements and the hazardous nature of the microbes and materials that they will handle throughout the semester. This information should be included in the syllabus for the class so it can be reviewed at any point during the course. The laboratory instructor must maintain student signed agreements in the laboratory. Alternatively, this may be performed and maintained online within Blackboard.
- Instructors must prepare, maintain, and post caution signs on laboratory doors (complete with biohazard symbol).
- Instructors must provide a detailed list of microorganisms that will be handled in the laboratory to students.
  This list may be included in the syllabus, a laboratory-specific biosafety manual, or online at the course website.
- Emergency phone numbers and information must be posted in the laboratory.
- Annual submission of course manual and list of microorganisms used in the laboratory to Biosafety Officer. Any major deviation from the material submitted must be updated and approved before the new semester as appropriate.

Biological Safety Level Two (BSL-2) Requirements

All information regarding biosafety and biosecurity should be included in the course syllabus. This will help ensure easy access to this important information at any time during the course.

BSL-2 General Requirements

BSL-2 laboratories are suitable for working with microbes posing a moderate risk to the individual and a low community risk for infection. There are many microorganisms handled in BSL-2 containment that may cause disease in humans via ingestion or inoculation. The BSL-2 requirements build upon those for BSL-1 facilities, and typically include additional engineering controls to protect students. Possible engineering controls include biological safety cabinets, centrifuge safety cups, and safety needle devices.

BSL-2 Laboratory Facility Requirements

- Non-porous floor (e.g., tile or epoxy), bench tops, chairs and stools*
- Sink for hand-washing
- Eyewash station
- Lockable door to the laboratory
- Proper pest control practices
- Separate storage area for personal belongings*
- Working and validated autoclave.
- Biohazard signage where cultures are used and stored (e.g., incubators), on the door to the room, and on containers used to transport cultures.
- An approved vendor must certify all biological safety cabinets used to handle infectious materials annually.

* It is understood that some current facilities may not be able to meet these requirements due to the original design of the laboratory space. Any facility renovation or new construction would need to include these requirements.
**BSL-2 Stock Culture Requirements**

- Stock cultures must be from approved and reputable sources.
- Subculturing microbes isolated from the environment, clinical samples, or other unknown locations is discouraged as BSL-2 (or higher) microbes may be isolated. Subculturing from the environment must be approved by the ASU IBC.
- Samples must never be obtained from clinical sites unless a full description of strain antibiotic susceptibility and resistance is provided, and the IBC has approved the use of these strains.
- Strains resistant to clinically relevant antibiotics must not be handled in teaching labs.
- Documentation must be maintained for all stock organisms, sources, and stock cultures.
- Fresh stock cultures of microorganisms must be obtained on a regular basis to be certain of the source culture, minimize spontaneous mutations, and reduce contamination.
- When possible, surrogates should be substituted for common pathogens (see the substitutes for ESKAPE pathogens provided in this document for recommendations).
- Stocks must be stored in a secure (locked) area.

**Common BSL-2 Microbes and Ordering Information from ATCC**

- When choosing a test organism, many instructors want to choose organisms that are clinically relevant (i.e., pathogens). There are six microorganisms that are considered major threats, not because they cause the most devastating illnesses but because they comprise the majority of antibiotic-resistant infections observed in health care settings. These are referred to as ESKAPE pathogens and include Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa, and species of Enterobacter (ESKAPE).
  - ESKAPE pathogen > Safe Relatives
    - Enterococcus faecium > Enterococcus raffinosus or Enterococcus casseliflavus
    - Staphylococcus aureus > Staphylococcus epidermidis
    - Klebsiella pneumonia > Escherichia coli
    - Acinetobacter baumannii > Acinetobacter baylyi
    - Pseudomonas aeruginosa > Pseudomonas putida
    - Enterobacter species > Enterobacter aerogenes

**BSL-2 Personal Protective Equipment Requirements**

- Safety goggles or safety glasses must be worn when handling liquid cultures, spread plating, or when performing procedures that may create a splash.
- Closed toe and heel shoes that cover the top of the foot must be worn.
- Wear long pants/long skirts (ankle length) to minimize potential for exposure to hazards.
- Lab coats (disposable or cloth) must be worn. Disposable coats may be reused but must be replaced on any sign of damage or degradation. Laboratory coats must be stored within the laboratory and must be assigned to individual students, not shared.
- Gloves must be worn when handling cultures, when staining microbes and when handling hazardous chemicals. Hands must be washed immediately after handling microbial cultures and anytime accidental contact occurs with the skin. Hand cleansing must be performed with soap and water, and if none is available with ethanol based hand sanitizer. Soap and water must be used as soon as possible if hand sanitizer is used.
BSL-2 Laboratory Work Practices

- Hands must be washed after entering and before leaving the laboratory.
- Long hair must be tied back.
- Long, dangling jewelry is not permitted in the laboratory.
- Benches must be disinfected upon entering the laboratory and at the end of the laboratory session. Any materials that are spilled must be immediately cleaned-up. Disinfectants used must be effective against microbes used in the laboratory. EH&S Biosafety and Biosecurity may be consulted for disinfectant recommendations.
- Food, water bottles, gum, and drinks of any kind are prohibited in the laboratory.
- Students should not touch their faces, apply cosmetics, adjust contact lenses, bite nails, or chew on pens/ pencils in the laboratory.
- All personal items must be stowed in a clean area outside the lab. Use of cell phones, tablets, and other personal electronic devices is prohibited unless students are required to use their cell phone cameras to record data to include in class presentations (e.g., biochemical test results, Gram stains). This photographic record is very helpful in troubleshooting experiments.
- Students are required to remove their gloves and wash their hands prior to handling their personal electronic equipment.
- Mouth pipetting is prohibited.
- All containers must be labeled clearly with the contents.
- The laboratory door must remain closed at all times when the laboratory is in session. The laboratory instructor must approve all persons entering the laboratory.
- Students must be taught proper technique to minimize production of aerosols. For example: when pipetting, place tip on side of tube and allow liquid to run down the side of the tube, and when flaring a loop to transfer culture, have a sterile agar plate used as a "sizzle" plate so students do not touch a culture with a really hot loop.
- All procedures that generate aerosols, such as centrifuging, grinding, blending, shaking, mixing, and sonicating must be performed inside a biological safety cabinet or using appropriate engineering controls (e.g., centrifuge safety cups). Biological safety cabinets must also be used when opening a container that may become depressurized when opened, and could release aerosols of the stock culture.
- Sharps usage must be minimized. Needles and scalpels are to be used according to ASU guidelines. All sharps (includes coverslips, slides and Pasteur pipets) must be disposed in a sharps container.
- Waste materials from the laboratory must be disposed properly.
- Test tube racks or other secondary containers must be used to move cultures in the lab.
- Stocks and other cultures must be stored in a leak-proof container when work is complete.
- Broken glass must be handled using a dustpan and broom or forceps and tongs. Students and laboratory personnel must not pick up broken glass with their hands. If contaminated, broom and other tools used to pick up broken glass must be disposed or sterilized.
- All spills or injuries must be immediately reported to the laboratory instructor. Spills or injuries must then be documented with the Biosafety Officer.
- Teach, practice, and enforce the proper wearing and use of personal protective equipment.
- Advise immune-compromised students and those living with or caring for an immune compromised person to consult physicians to determine the appropriate level of laboratory participation. Students should not be asked to reveal if they are immuno-compromised. A
general announcement should be made that students with a reduced immune status should consult with ASU Health Services. A note from ASU Health Services is sufficient to excuse a student from laboratory work.

- Lecture should be performed before materials are brought to the work areas. Note taking should be kept to a minimum when hazardous materials are out. When possible, note taking should be conducted away from the work area and after gloves have been removed.
- Use micro-incinerators rather than Bunsen burners. Bunsen burners are not permitted in biological safety cabinets. Micro-incinerators may also be used to heat fix bacterial smears on microscope slides and flaming the end of a test tube by passing these items over the entrance to the micro-incinerator.

BSL-2 Training Practices
- Faculty and teaching assistants must complete all required safety trainings prior to beginning the first day of class and annually thereafter.
- Instructors and/or teaching assistants must review basic biosafety and microbiological practices with students on the first day of the laboratory. The requirements listed in this document should be included in this training session. The training session must be documented with a sign-in sheet maintained by the instructor.

Arizona State University Institutional Biosafety Committee
- Students and instructors are required to handle microorganisms safely and in conjunction with requirements outlined in the MSU Biosafety Manual.
- Students must be informed of safety precautions applicable to each exercise before the procedure is performed.
- Students must demonstrate proficiency in standard aseptic technique and BSL-1 practices before allowing them to work at BSL-2.

BSL-2 Documentation
- Safety Data Sheets (SDS) must be available for all chemicals in the laboratory.
- Students must sign safety agreements indicating that they have been informed about the safety requirements and the hazardous nature of the microbes and materials that they will handle throughout the semester. This information should be included in the syllabus for the class so it can be reviewed at any point during the course. The laboratory instructor must maintain student signed agreements in the laboratory. Alternatively, this may be performed and maintained online in Blackboard.
- Instructors must prepare, maintain, & post caution signs on lab doors (with biohazard symbol).
- Instructors must provide a detailed list of microorganisms that will be handled in the laboratory to students. This list may be included in the syllabus, a laboratory-specific biosafety manual, or online at the course website.
- Emergency phone numbers and information must be posted in the laboratory.
- Annual submission of course manual and list of microorganisms used in the laboratory to the IBC. Any major deviation from the material submitted must be updated and approved before the new semester as appropriate.
- All work at BSL-2 must be registered with the Institutional Biosafety Committee.
- All requirements for BSL-2 must be followed as outlined in the Biosafety Manual.