Laboratory Biosafety Manual

Building/Lab Room No(s):

Biosafety Containment level: BSL

Date: Click here to enter text.

Expires One year from the above date.
**PI's Last Name Laboratory**

**Building(s) and room number(s)**

**Note:** Put this document in a loose-leaf binder so that other information can be easily added and updated.

This template is provided to assist Principal Investigators (PIs) in the development of a laboratory-specific biosafety manual with instructions to safely handle and manipulate biological agents in the laboratory. The PI is responsible for including basic background information for each agent, making protocols (IBC, IACUC etc) available, writing an exposure risk, detailing surface decontamination, and writing standard operating procedures (SOPs) for experiments where safety is a concern.

Please provide lab-specific information where you see gray text fields. Training dates should be maintained in the provided table. Additions/changes that may be helpful to lab personnel are encouraged. If any laboratory determines the need to deviate from standard BSL2 work practices discussed in this manual, then these alterations along with a written explanation must be submitted to the Biosafety Officer (BSO) for approval.

In addition to this manual, the National Institutes of Health (NIH) and the MSU Institutional Biosafety Committee (IBC) require the lab to follow BSL procedures as outlined in the **BMBL, 5th Edition**. For research involving recombinant DNA, the lab must also follow the **NIH Guidelines for Research Involving Recombinant DNA Molecules**.

Prior to working in the lab, personnel must read this entire manual, sign & date below. By signing this page, lab personnel agree to abide by the safety precautions and procedures discussed herein.

_I have read, understand, and agree to adhere to the biosafety procedures contained within:_

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Responsibilities

Principal Investigator (PI) Responsibilities
Dr. Click here to enter text has the primary responsibility for ensuring that the laboratory is safe. Dr. Click here to enter text is also responsible for the safe use of biological materials used in the lab.

In addition, Dr. Click here to enter text is responsible for the following:

• Limit personnel, student, and visitor exposure to hazards to the lowest practical level.
• Be familiar with the required medical surveillance for each type of biological agent used in the laboratory.
• Develop written lab-specific safety procedures and train personnel on them.
• Maintain documentation of training in this Biosafety Notebook.
• Provide the appropriate Personal Protective Equipment (PPE) and instruction on proper use.
• Ensure all forms of waste are properly disposed of.
• Report spills, exposures or incidents to Phil J. Merta, Biosafety Officer, at 994-3779.

Laboratory Staff/Student Responsibilities

• Complete all required training before conducting any lab activity.
• Knowledge of the biological agents and procedures used in the laboratory.
• Follow approved lab procedures and safety guidelines.
• Know emergency procedures.
• Report any unsafe conditions to the PI and/or the Biosafety Officer.
• Utilize appropriate lab equipment and containment facilities.

General Emergency Information

Emergency Contacts

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<tr>
<th>Biosafety Officer</th>
<th>Phil J. Merta</th>
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<tr>
<td>Office Phone:</td>
<td>994-3779</td>
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<td>Cell Phone:</td>
<td>406-600-8787</td>
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| IBC Protocol #(#s):     | Enter number(s) |
| IACUC Protocol #(#s) (if applicable): | Enter number(s) |

If there is an emergency, call x2121 to reach MSU Police Department.

If there is a fire or explosion immediately pull the fire alarm and evacuate the building. Then call x2121 from a safe location.

If any emergency or significant spill/exposure occurs in the laboratory, immediately notify the Biosafety Officer and your lab supervisor/PI.
**Fire Alarms/Extinguishers**

Locations of fire pull station alarms:

Click here to enter text.

Location of fire extinguishers:

Click here to enter text.

Know the location of each of these, and identify the location of the extinguisher closest to your lab bench. If the fire alarm sounds, leave the building immediately and move away to a safe distance.

**Eyewash and Emergency Shower Stations**

Location of eyewash:  
Click here to enter text.

Location of shower:  
Click here to enter text.

In case of exposure, proceed to nearest eyewash station. Hold eyelids open with thumb and forefinger and rinse for at least 15 minutes. Wash from outside edges towards the inside to prevent washing back into the eye.

Rinse should be aimed at the inner corner of the eye (near the nose) not directly at the eyeball. “Roll” eyes around and up and down to ensure full rinsing.

Contact lenses (if worn) should be removed as soon as possible. Have another member of the lab call for emergency response immediately. The area around the eye wash station must always remain clear.

Please flush and document eyewash stations on a weekly basis so that they are clean and ready in case of an exposure event.

**Standard Operating Procedures**

**Standard Microbiological Practices**

These refer to the safe laboratory work practices when experimenting with biological agents.

**Hygiene and Housekeeping**

Keep work areas clean and uncluttered to reduce the chance of cross-contamination and inadvertent exposure to biohazards. To avoid ingestion of contaminated material, use a mechanical pipetting device, keep food out of refrigerators and microwaves in work areas, eat, drink, or apply cosmetics only in designated “clean” areas outside the laboratory.

1. Wash hands with soap and water after removing gloves, before leaving the lab, and when handling materials known or suspected to be contaminated.
2. Clean work surfaces and decontaminate with Click here to enter text at the end of each day.
3. Remove gloves before leaving the lab, touching the face, keyboards, or control panels, and before using the elevator.
Personal Protective Equipment

The following PPE must be worn when working with agent(s):

*Please check appropriate boxes by double clicking and selecting “checked.”*

- Gloves
- Latex
- Nitrile
- Safety glasses
- Face shield
- Lab coat
- N95 Respirator
- Surgical mask
- Shoe covers
- Medical scrubs
- Hair net

**PPE Considerations**

a. Wear gloves if skin on the hand is broken, if rash is present, and when handling biological waste.

b. Remove rings or other jewelry that could puncture gloves.

c. Wear the appropriate glove for the hazard. Usually a type of latex or nitrile glove is recommended for working with biological material.

d. Avoid reusing gloves unless they can be decontaminated.

**Wear the eyewear appropriate for the hazard:**

1. Approved for UV light or other rays that could damage eyes.
2. Standard lab safety glasses, googles, or face shield when performing operations that may potentially generate aerosols.

**SOPs for Dr. Last name Laboratory when using BSL2 Agents**

The purpose of this section is to develop SOPs that specifically outline instances during protocols where consideration for safety with a BSL2 agent is paramount. Detailed, step-by-step protocols describing entire experiments with materials and methods are not necessary. Examples of SOPs where safety is emphasized are bulleted below:

- Propagation of viruses
- Experiments that require PPE in addition to a lab coat and gloves
- Experiments that require manipulation of a BSL2 agent outside a biosafety cabinet
- How to properly vortex or sonicate a viable BSL2 agent
- Safety concerning the handling of human or non-human primate cell lines or tissues
- Safety when injecting a research animal with a BSL2 agent
- How to safely centrifuge a sample containing BSL2 agents

Please enter SOPs under separate headings.

*Click here to enter text.*

*Place copies of all SOPs, approved IBC, IACUC, and IRB protocols after this page.*
DEALING WITH INJURIES

Injury to an individual in the laboratory (i.e. needle stick, cut, biological/chemical exposure incident – splash, etc.):

a. Immediately stop work and flush affected area with soap and water for 15 minutes.
b. If the injury is a Medical Emergency call 911 or campus police x2121.
c. Secure all infectious materials.
d. Notify Dr. Click here to enter text. and Biosafety Officer at x3779 or Campus police x2121 after working hours. This is a very important as the University maintains an ongoing log/list of spills and injuries and as applicable reports these as required under the NIH Guidelines for Research Involving Recombinant DNA Molecules.
e. Use the nearest First Aid Kit located in the laboratory.
f. If during working hours seek medical attention at the Bridger Occupational Health (3400 Laramie; Bozeman). After working hours seek medical attention at the Bozeman Deaconess Hospital Emergency Room (915 Highland Blvd; Bozeman).
g. Dr. Click here to enter text. will complete the Workers Compensation Injury/Incident Report form documenting the route of exposure and the circumstances under which the incident occurred.

ACCIDENTAL NEEDLE STICK: EMERGENCY SOP

To report any injury:  http://firstreportinjury.mus.edu

If you sustain a needlestick or sharps injury:

- Encourage bleeding at the site of puncture.
- Wash the wound immediately with soap and water.
- Dry and cover the wound.
- Flush away any splashes of blood & needle contents on other parts of your body with water.
- Seek prompt treatment.
- Report the incident as soon as possible!
- Discuss the risk of infection based on the circumstances of the injury.

- Determine whether HIV or other disease exposures are possible. (HIV 0.03% serconversion rate from needle sticks). If warranted begin antiviral therapy within 2 hours.

Laurie Shute - Occupational Health Manager
Work: 406-994-7384   Cell: 406-570-7812
Bridger Orthopedic West- MSU Occ. Healthcare provider
3400 Laramie Dr, Bozeman, MT 59718
406-586-5694 Open M-F 7am-6pm/S-S 9am-5pm
BIOLOGICAL SPILLS IN THE LABORATORY

Call the Biosafety Officer when a significant spill occurs. A lab incident report form must be filled out for significant spills.

A significant spill is defined as:

- Spills greater than 5 ml outside primary containment.
- Spills that result in an exposure.
- Spills that present an inhalation hazard.
- Spills that cannot be easily cleaned.
- Spills that endanger people or the environment.

**Biological Spill Kit**
Location of spill kit: Make sure that the location is prominently marked and easy to find.

Click here to enter text.

Note that 10% bleach must be made up weekly; other more stable disinfectant solutions (e.g. 5% Microchem) may be considered to extend the shelf life of your spill kit.

Refer to Biohazardous Spill Cleanup SOP on Next Page.
BIOHAZARDOUS SPILL CLEANUP SOP

Spill Involving BSL1 Material

Clear area- alert other lab occupants of spill and set up a safety perimeter.

1. Put on Personal Protective Equipment (PPE: gloves, lab coat, and eye protection).
2. Remove contaminated sharps from spill using forceps or tongs.
3. Cover the spill with paper towels or other absorbent material.
4. Carefully pour bleach (1:10 dilution) around the edges of the spill and work from the edges to the center. Allow for 30-minute exposure time.
5. Place towels in a biohazard bag for disposal.
6. Clean spill area with fresh towels soaked in disinfectant.
7. Wash hands and any potentially contaminated skin before exiting the laboratory.
8. Dispose of all materials including gloves in biohazard bag; autoclave when convenient.
9. Alert your supervisor and Biosafety Officer (Phil J. Merta 994-3779).

• If spill is inside a BSC – Keep BSC running for at least 15 minutes after the cleanup. Clean spill tray below work area and trough below air intake grill while BSC is running.

Spill Involving BSL2 Material

1. Alert people in immediate area of spill; set up safety perimeter to prevent spread of contaminants.
2. Keep area clear of all personnel for at least 10 minutes to allow aerosols to settle.
3. Put on PPE (gloves, eye protection, and lab coat).
4. Remove contaminated sharps from the spill using forceps or tongs.
5. Cover the spill with paper towels or other absorbent material.
6. Carefully pour bleach (1:10 dilution) around the edges of the spill and work from the edges to the center.
7. Allow 30-minute exposure time of disinfectant.
8. Discard paper towels or absorbent material into biohazard bag.
9. Clean with fresh paper towels soaked in disinfectant.
10. Wash hands and any potentially contaminated skin before exiting the laboratory.
11. Dispose of gloves and other potentially contaminated materials in biohazard bag.
12. Notify your supervisor and Biosafety Officer (Phil J. Merta 994-3779).

• If spill is inside BSC- Keep BSC running for at least 15 minutes after the cleanup. Clean
spill tray below work area and trough below air intake grill while BSC is running.

Preparing a Biohazard Spill Kit

Every biosafety laboratory that works with biological agents must have a biohazard spill kit on hand, that is readily accessible and easy to find in the laboratory. It should have appropriate equipment and supplies on hand for managing spills and accidents involving biohazardous materials. Biosafety engineering equipment in the lab should include an eyewash station, a hand-washing sink with soap and paper towels, and a shower. A biohazardous spill kit should also be kept on hand. The supplies available in a biohazard spill kit should include, but are not limited to:

1. An autoclavable plastic bucket or bin to keep all contents in.
2. A copy of this Spill Cleanup Protocol.
3. PPE - Nitrile disposable gloves, eye protection, lab coat (nearby if not in kit).
4. N95 dust mask respirator(s).
5. Disposable shoe covers (booties).
6. Absorbent material, such as paper towels.
7. All-purpose disinfectant, such as normal household bleach (freshly diluted 1:10).
8. Tongs and/or forceps, and/or dustpan and hand broom for cleaning up broken glass or other contaminated sharps.
9. Sharps waste container (in lab, nearby).
10. Autoclavable biohazard waste bags.
11. Biohazardous spill warning signs.

All non-disposable items should be autoclavable or compatible with the disinfectant to be used. Most of the listed items, as well as other biohazard spill control items, are available at Central Stores, and often are contained within various commercially-available biohazardous spill control kits.

*******************************************************************************

10 | P a g e
Biological Waste Disposal

Liquid Biohazardous Waste Disposal:
All liquid biological waste from the lab must be treated prior to disposal. Examples of biological waste include cell lines, recombinant DNA, recombinant proteins, biological agents, and any associated media or buffer.

The procedures below outline the steps to take to treat liquid biohazardous waste generated in Dr. lab:

a. Always wear appropriate PPE such as disposable gloves, lab coat and eye protection (safety glasses or goggles) when working with biohazardous waste.
b. When liquid biohazardous waste is anticipated to be generated, add 100 ml of undiluted bleach into a 1-liter beaker. Label the beaker appropriately.
c. As experiments are performed and completed pour the biological waste into the beaker with the bleach.
d. When experiments are completed (and if the beaker contents are less than 1-liter) add water to bring the volume to 1-liter. This provides for a 10 % bleach solution.
e. Let 10 % bleach and biological waste solution stand for at least 1 hour.
f. Dispose of the solution with care to avoid splatter or spillage, down the lab sink.
g. Rinse the beaker thoroughly with water.

Solid Biohazardous Waste Disposal:
The procedures below outline the steps to treat solid biohazardous waste generated in Dr. lab:

a. All solid lab waste that has come in contact with biological waste must be treated prior to disposal. Examples include used PPE, paper towels, pipette tips, Petri dishes, pipettes, culture flasks.
b. Always wear appropriate PPE such as disposable gloves, lab coat, and eye protection (safety glasses or goggles) when working with biohazardous waste.
c. Place all potentially contaminated items in biohazardous waste bag.
d. Once the bag is ¾ full close bag and place autoclave tape on the bag.
e. Take the biohazard bag to the autoclave room per your buildings transportation guidelines as follows and place in the autoclave.
f. Complete autoclave log book entry and autoclave biohazardous waste for following manufactures’ recommendations for autoclave operation.
g. Once the autoclave cycle is complete the load within has been sterilized if the autoclave tape has turned color and the autoclave display shows no errors. Allow sterilized bag to cool in a bin.
h. Place the biohazardous bag into a black garbage bag prior to disposing in the dumpster.
i. If the autoclave tape did not turn color and/or the autoclave display indicates errors occurred during operation or an incomplete cycle the load has not been sterilized. Notify at as the load is still considered biohazardous.

Sharp Biohazardous Waste Disposal:
The procedures below outline the steps to carefully handle sharps in Dr. lab:

a. All used sharps must be immediately discarded into a sharps container.
   a. Sharps containers must be kept upright and never overfilled- see “fill-line”.
   b. Put sharps containers in immediate work area for easy use.
b. When sharps waste has reached the pre-marked “fill-line” of the sharps container close and lock lid.
c. Contact Safety and Risk Management to have sharps containers picked-up and replaced.
Do not re-cap needles!!

Containers
Sharps go in dedicated waste

Good laboratory practices

Autoclave, submit to SRM
Seal, mark with tape
Proper Biohazard Waste Disposal

Gloves
Pipet tips
NO SHARPS

Biohazard waste

Seal bag loosely.
Mark with tape.
(150-180°C up to 2h)
Autoclave

Take out, let cool.
Put into regular
dark garbage bag
Normal waste stream.

P a g e
Hazardous (Chemical) Waste Management Guidelines

Please contact Safety and Risk Management (SRM) to dispose of hazardous chemical waste materials.

Disposal Guidelines: [http://www.montana.edu/srm/chemical-safety/disposalguidelines.html](http://www.montana.edu/srm/chemical-safety/disposalguidelines.html)

1. **NO hazardous waste** should ever go down any MSU drain, at any time or in any amount.
2. Hazardous waste liquids are collected by SRM using the waste pickup request link at: [http://www.montana.edu/srm/forms/waste/](http://www.montana.edu/srm/forms/waste/)


Hazardous Materials Contacts:

- **Safety & Risk Management**
  - Ryan Brickman, - Chemical Safety Officer x7760
  - Patrick Ryan, - Hazardous Materials Manager x7803.

Labels available from SRM; or print this page and use this blank form:

![Hazardous Waste Label Diagram](http://www.montana.edu/srm/forms/waste/)

Submit Request for Pickup at: [http://www.montana.edu/srm/forms/waste/](http://www.montana.edu/srm/forms/waste/)
Inactivation and Surface Decontamination

Describe the reagents (disinfectants) and/or processes used to inactivate the agent(s) and the method to decontaminate surfaces.

Decontamination (decon)

Decontamination- Any procedure that reduces pathogenic microbes to a level where items are safe for handling and disposal

**Cleaning** – A process that removes foreign material (dirt, organic matter)
Usually done with soap and water or detergent

- **Disinfection** – Destruction of most but not necessarily all pathogenic microbes or their spores.
  Generally done with Chemicals (bleach, ethanol)

- **Sterilization** – Killing of all living forms of microbes including spores
  Autoclave
Training

All laboratory research personnel must take institutional provided training. Training must be documented. Personnel should not initiate research until training is completed. Attach copies of completed training certificates below.

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<th>Lab Personnel</th>
<th>Relevant Training Dates</th>
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<tbody>
<tr>
<td>Name</td>
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<td>Mark MSU or CITI + date</td>
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Agent(s)-specific Training. Laboratory personnel are not allowed to work with agent(s) until they have been trained by the PI who supervises their work, or a designated technical expert. The worker should demonstrate good microbiological skills and an understanding of this SOP prior to being permitted to work with agent(s).
Training Requirements & Documentation

Requirements:

**Biosafety for BSL1 and BSL2 Laboratories training** - Required for all researchers; **3-year certification**.

**NIH Recombinant DNA (rDNA) Guidelines** - Required for all researchers working on rDNA; **3-year certification**.

**OSHA Bloodborne Pathogens** - Required for any work with human-sourced material; **1-year certification**.

**Hazard Communication** - Required for all employees that work with/around Hazardous Chemicals (cleaning and industrial chemicals, laboratory chemicals, solvents or acids, mechanical fluids, etc.) or anyone working with human-sourced material, i.e. body fluids of any kind, tissues, or cell lines; **1-year certification**.

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**In House Training:** **Biosafety & Hazard Communication Training**

*This is a 2-hour course that covers ALL THE TRAINING REQUIREMENTS.*

Details: [http://www.montana.edu/orc/biosafety/training.html](http://www.montana.edu/orc/biosafety/training.html)

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**Online Options:** **Collaborative Institutional Training Initiative (CITI Program) website:**

[https://about.citiprogram.org/en/homepage/](https://about.citiprogram.org/en/homepage/)

Instructions: [http://www.montana.edu/orc/documents/ibc/How%20to%20access%20CITI%20Program%20training.pdf](http://www.montana.edu/orc/documents/ibc/How%20to%20access%20CITI%20Program%20training.pdf)

**Hazard Communication** Online training option: (via SRM)

Biohazard Warning Signs and Posting

Each laboratory must clearly display a sign that provides safety information to visitors and service personnel. ORC/SRM will provide the signs.

a. All areas and laboratories which contain biohazardous agents must be posted with a biohazard sign.
b. The sign must have information regarding biosafety level, materials used, entry requirements, exit requirements, emergency contact name and phone number.

Please confirm that your laboratory entry signs are accurate and up to date. If they need correction, please contact Phil Merta (BSO x3779) or Ryan Brickman (CSO x7760).

Biological Safety Cabinets (BSCs)

There is no requirement for directional inward airflow in a BSL-1 or BSL-2 laboratory, except as may be required for chemical odor control.

BSC’s should be positioned in the laboratory away from normal traffic patterns to minimized airflow disruption.

Some work may be done on the open bench by persons wearing appropriate protective clothing or gear. Any work that may produce splatters or aerosols of infectious materials should be done inside a biological safety cabinet (BSC).

Before materials are introduced into the BSC, they should be wiped with disinfectant to remove any external contaminants.

Clean materials should be kept to one side of the work surface, dirty items on the other. Management of workflow within the BSC is crucial to preventing cross-contamination.

Rapid air movement outside the cabinet (caused by co-workers walking past, air supply vents directed across the face of the BSC, etc.) will interrupt the rather fragile air curtain, which may cause air-borne contaminants in the cabinet to be drawn into the lap of the worker.

The chair should be adjusted so that the lower portion of the sash is even with the worker’s armpits.

Any paper or plastic materials introduced into the BSC should not be allowed to interfere with air flow through the front or rear grilles.

The downward airflow from the supply filter “splits” about one third of the way into the cabinet; in the front third, air moves to the front grille, with the remainder of the air flowing to the rear. This means that aerosol-generating activities should be performed towards the rear of the cabinet to provide further worker protection.

Date of last Biosafety Cabinet Recertification: [Click here to enter text]
Agent(s)-Specific Pathogen Safety Data Sheets

Pathogen Safety Data Sheets can be found at the MSU Biosafety website (http://www.montana.edu/orc/biosafety/psds.html) or, if needed, the Public Health Agency of Canada website (http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php)

Exposure Risk
Describe how laboratory personnel could be exposed to the agent(s). Include practices that pose potential for exposure, such as those that could create aerosols.

Click here to enter text.

Place your specific Pathogen Safety Data Sheets for each Biological Agent used in Dr. Laboratory in the notebook behind this page

LIST of YOUR BIOAGENTS:

Click here to enter text.

Print out copies of the PSDS sheets and put them after this page.