



# Laboratory Biosafety Manual

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# **INTRODUCTION**

## **PURPOSE**

This manual defines the biological safety policies and safe practices for laboratories working with biological organisms and/or biological materials at Michigan Technological University. This manual may be adopted directly by Faculty / Principal Investigators for use in their teaching or research laboratory or sections of this manual may serve as a resource in creating a Biosafety manual that is specific for the needs of their laboratory.

## **POLICY**

As an institution of research and higher education, Michigan Technological University is committed to maintaining a safe working environment in all research and teaching laboratories where biological materials are used. As the foundation of that commitment, the University complies with all federal and state regulations and guidelines governing the use of biological materials in the laboratory. For specific information concerning these regulations and guidelines see:

[NIH Guidelines for Research Involving Recombinant DNA Molecules.](#)

[Biosafety in Microbiological and Biomedical Laboratories \(BMBL\)](#)

[MIOSHA: Bloodborne Infectious Diseases](#)

# **RESPONSIBILITIES**

## **OFFICE OF RESEARCH INTEGRITY AND COMPLIANCE**

1. Requires registration of all research and teaching programs involving human subjects, animals, recombinant DNA and other biological organisms and materials; and ensures University compliance with all federal and state regulations and recommendations pertaining to research in these areas.
2. Establishes policies for the safe conduct of research that protect the health of university employees, the community and the environment from exposure to potentially harmful biological materials.
3. Determines the necessity for health surveillance of personnel involved in research with recombinant DNA or other biological organisms/materials.
4. Assists Principal Investigators in complying with regulations and guidelines that are applicable to their research.
5. Establishes an Institutional Biosafety Committee (IBC); appoints members to serve on the committee and ensures that the committee has adequate expertise and training.

6. Reports any significant problems or violations of NIH guidelines and any significant research-related accident or illness to NIH/OBA.

## **INSTITUTIONAL BIOSAFETY COMMITTEE (IBC)**

The institutional biosafety committee is comprised of no fewer than five members with at least two members who are not directly affiliated with the University and represent the interests of the surrounding community. It is recommended by NIH that the committee include members with expertise in biological safety and physical containment, recombinant DNA technology, animal or plant research and have available consultants with knowledge of University policies, applicable laws, standards of professional conduct, etc. Collectively members of the committee shall have the expertise and capability to assess the safety of research involving recombinant DNA or other biological organisms and materials and identify any potential risks to individual and public health and to the environment.

Responsibilities include:

1. Review of all research that is conducted at or sponsored by the University involving recombinant DNA research subject to the NIH Guidelines and biological research requiring biosafety containment levels greater than BSL-1. This review will include:
  - a. Independent assessment of the risks associated with the research and verification of containment levels assigned by the PI.
  - b. Assessment of facilities, equipment, procedures, practices, training and all other elements associated with the research.
2. Notification of the Principal Investigator of the committee's actions.
3. Periodic review of recombinant DNA and biological research.
4. Adopt emergency plans for accidental spills, personnel contamination, loss of containment and research related illnesses.
5. Keep a record of meetings, providing sufficient detail to serve as a record of major points of discussion and the committee's rationale for particular decisions, documenting that the IBC has fulfilled its review and oversight responsibilities.
6. Reports any significant problems or violations of NIH guidelines and any significant research-related accident or illness to NIH/OBA.
7. File an annual report with the NIH

## **BIOLOGICAL SAFETY OFFICER**

1. Assists faculty members with establishing and maintaining a safe working environment in both research and teaching laboratories.

2. Serves as a member of the University's Institutional Biosafety Committee.
3. Reports to the IBC and the University's Office of Research Integrity and Compliance any significant problems, violations of the *NIH Guidelines*, and any significant research-related accidents or illnesses unless a report has already been filed by the Principal Investigator.
4. Conducts laboratory inspections to ensure that standards and containment conditions established by the Institutional Biosafety Committee are followed.
5. Develops emergency plans for handling accidental spills and personal contamination and investigates laboratory accidents involving recombinant DNA and other biological agents.
6. Provides technical advice to Principal Investigators and the Institutional Biosafety Committee.
7. Works with departmental laboratory coordinators to facilitate compliance with regulatory guidelines and University policies.

## **PRINCIPAL INVESTIGATOR**

The Principal Investigator is responsible for full compliance with University policies and all government regulations and guidelines that are applicable to their research. Additional responsibilities include:

1. Registration of teaching and/or research laboratory activities with the office of Research Integrity and Compliance (RIC).
2. Conduct a laboratory risk assessment with assistance as needed from the biosafety officer that
  - a. Describes the research activities in your laboratory.
  - b. Identifies the organisms and/or biological materials used in the laboratory and their recognized risks.
  - c. Describes the risks associated with laboratory procedures and the implementation of appropriate controls and/or practices to minimize those risks.
  - d. Evaluates the experience and training of individuals working in the laboratory.
3. Ensure that practices to minimize risks are written into the laboratory's standard operating procedures.
4. Ensure that all laboratory personnel understand the risks connected with work in the laboratory; have received appropriate training to mitigate those risks and follow the required safety practices in their work.
5. Ensure that emergency plans for spills and/or personal exposures as set by the Institutional Biosafety Committee are followed in the laboratory.

6. Verify the performance of all safety equipment used in the laboratory including PPE, biosafety cabinets, aerosol proof centrifuges, etc
7. Report any significant problems, violations of the *NIH Guidelines*, or any significant research-related accidents and illnesses to the Biological Safety Officer (where applicable), Greenhouse/Animal Facility Director (where applicable), Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities (if applicable) within 30 days.

## LABORATORY STAFF

1. Follow established laboratory safety practices and standard operating procedures.
2. Verify the performance and safety of all equipment before use. This includes Personal Protective Equipment (PPE), biosafety cabinets, centrifuges, fume hoods, etc.
3. Communicate to the Principal Investigator any unsafe practices or conditions in the laboratory.
4. Report any spills or accidents involving biological materials to the Principal Investigator.
5. Inform the Principal Investigator of any changes in your health status that may be related to your work in the laboratory or that may affect your susceptibility to exposure to materials used in the in the laboratory.

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## GENERAL INFORMATION

### Laboratory Access

- 1 The Principal Investigator or laboratory supervisor authorizes access to the laboratory and is responsible for the safety of individuals working in the laboratory.
- 2 Individuals who make requests to use the laboratory or laboratory equipment and who are not directly affiliated with the lab must be advised of the potential risks associated with the laboratory and receive training appropriate to the work that will be performed.
- 3 Visitors must be accompanied by an individual with authorization to work in the laboratory.
- 4 Children under age 16 are not permitted in laboratories. Exceptions may be granted for supervised youth participating in University sponsored programs.
- 5 The doors of unoccupied laboratories will be locked, to prevent unauthorized access.
- 6 The increased risks to individuals undergoing immunosuppressive therapy, pregnant women, etc., must be evaluated and addressed before they enter or work in laboratories where dangerous materials such as infectious agents, toxins or radioactive materials are used.

## **Signage**

A “caution” sign will be posted on or near the entrance door to biological laboratories. The sign shall include the following information:

1. The biosafety level of the laboratory.
2. A listing of the hazards associated with work in the laboratory (infectious agents, toxins radioactive materials, etc.,).
3. The name(s) and contact information of the Principal Investigator, laboratory supervisor and /or other responsible persons.

In addition to cautions posted at the entrance to the laboratory, appropriate universal warning signs or symbols shall be placed on all freezers, refrigerators, centrifuges, incubators, waste containers, etc., where hazardous materials are used or stored.

## **Training**

Training for individuals working in the laboratory must be appropriate for their duties and responsibilities. Training must be provided prior to beginning work in the laboratory and refreshed annually. At a minimum training will consist of practices to minimize exposure to hazardous materials including the appropriate use of personal protective equipment and the use of laboratory equipment such as centrifuges, biological safety cabinet, fume hood, autoclave, etc. The University is a member of the Collaborative Institutional Training Initiative (CITI) that provides for standardized training modules in Biosafety, The Use of Human Subjects in Research and Laboratory Animal Use and Care.

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## **APPENDICES**

### **BIOLOGICAL SAFETY CABINETS (BSC)**

#### **Types of Biological Safety Cabinets**

When properly installed and certified, the biological safety cabinets (BSC)s listed in the table below may be used for work in BSL 1, BSL 2 and BSL 3 laboratories. As indicated these BSCs provide varying degrees of protection when working with infectious or toxic materials. Small or minute amounts of volatile chemicals (as indicated in the table below) may only be used if the BSC is exhausted to the outside through an in-line charcoal filter and is equipped with an explosion proof motor and electrical components. **BSCs DO NOT provide the same level of protection as a chemical fume hood when working with volatile or other hazardous chemicals and should not be used as a substitute for a chemical fume hood.** Similarly, a chemical fume hood will not provide adequate protection when working with infectious agents and other hazardous biological materials.

BSC Class	Airflow pattern	Notes concerning specific uses
<b>Type I</b>	Air flow in at the front and is exhausted through a HEPA filter.	<ul style="list-style-type: none"> <li>Material in BSC is not protected, provides protection only to personnel and environment.</li> <li>Can be used with non volatile toxic chemicals and radionuclides and when exhausted outdoors may be used with volatile chemicals</li> </ul>
<b>Type II A1</b>	70% of air is re-circulated in cabinet and 30% is exhausted through a HEPA filter either to the room or through a canopy to outside.	<ul style="list-style-type: none"> <li>Do not use with volatile chemicals. With 70% recirculation, levels of volatile chemicals can reach unsafe levels.</li> <li>Only minute amounts of non-volatile toxic chemicals and radionuclides may be used.</li> </ul>
<b>Type II A2</b>	Similar to Type II, A1, but has 100 lfm intake air velocity and plenums are under negative pressure to the room; exhaust air can be ducted to the outside through a canopy unit.	<ul style="list-style-type: none"> <li>Suitable for use with non-volatile toxic chemicals and radionuclides.</li> <li>Can be used with minute amounts of volatile chemicals if ducted to the outside through an exhaust canopy.</li> </ul>
<b>Type II B1</b>	30% of air is re-circulated and 70% is exhausted. Exhaust cabinet air must pass through a dedicated duct to the outside through a HEPA filter.	<ul style="list-style-type: none"> <li>Suitable for use with non-volatile toxic chemicals and radionuclides.</li> <li>Can be used with minute amounts of volatile chemicals.</li> </ul>
<b>Type II B2</b>	No air recirculation; total exhaust to the outside through a HEPA filter.	<ul style="list-style-type: none"> <li>Suitable for use with non-volatile toxic chemicals and radionuclides,</li> <li>Can be used with volatile chemicals in small amounts.</li> </ul>

## Working in a Biological Safety Cabinet

In addition to the high efficiency particulate air (HEPA) filters that remove particles of 0.3 µm with at least 99.97% efficiency, the protection provided by a BSC is also dependent upon an undisrupted, directional airflow within the cabinet. Disruptions to the airflow resulting from inappropriate work practices or laboratory design can marginalize the operation of the BSC and put the user at risk. For this reason, the BSC should be located away from doorways, high traffic areas and other locations in the laboratory where equipment or the movement of people may generate air currents that disturb air flow in the cabinet.

### ***Preparing for work in the BSC***

1. Before starting work in the BSC, review all procedures that will be used; identify the necessary equipment and materials that will be needed and develop a plan for safe and efficient work.
2. If the cabinet is not running, turn on the blower and fluorescent lights and turn off the UV light if it is on.
3. Verify that the BSC is operating correctly:
  - a. Check the instrument display/gauges for operational status.
  - b. Check the intake and exhaust grills for obstructions.
  - c. Check that the sash is in the appropriate position.
  - d. Check for the inward flow of air at the face of the BSC by holding a tissue near the bottom edge of the sash.
4. Wipe down the interior surfaces of the cabinet with an appropriate disinfectant such as 70% ethanol, WEX-CIDE, a 1:100 dilution of household bleach (0.05% sodium hypochlorite) or another suitable disinfectant. Note that bleach, although an excellent and inexpensive disinfectant, will react with stainless steel surfaces of the hood and must be followed with a rinse of sterile water or 70% ethanol.
5. Load the cabinet with materials that will be needed for the procedure, wiping their surfaces with 70% ethanol to minimize the introduction of contaminants into the BSC. Position the materials near the back of the hood and organize them in a manner that will allow for the separation of clean and contaminated items during your work in the hood. Only materials needed for immediate work should be placed in the cabinet. Extra supplies (gloves, culture flasks/plates should be stored outside the cabinet).
6. Define a work area 4 to 5 inches behind the front grill of the hood. This area may be covered with a plastic-backed absorbent liner to minimize the effects of splatter and aerosol generation and to facilitate clean-up in the event of a spill. The liner may be moistened with an appropriate disinfectant to promote aseptic conditions within the cabinet.
7. Before beginning your work, allow the hood to run for a minimum of 5 minutes to purge any airborne contaminants from the work area.

### ***Working in the BSC***

1. Wear appropriate personal protective equipment (PPE). At a minimum, a lab coat with close-fitting sleeves and gloves should be worn. Because it is appropriate to wash your hands after removing gloves, double-gloving is a good option if you anticipate the need for glove changes during your work or in the event of a spill a double pair of gloves adds an additional layer of personal protection.
2. Proper aseptic technique is essential. The BSC will prevent aerosol contamination but will not prevent contact transfer resulting from poor technique.

- 3 Avoid rapid, sweeping movements of the arms into or out of the cabinet. Move items into or out of the cabinet slowly and perpendicular to the face of the cabinet to minimize disturbance to the protective curtain of air.
  - 4 Do not block the air flow in the BSC by resting your arms or placing discarded plastic wrappers, procedure notes or other materials on the grill at the front of the BSC.
  - 5 Organize your work to maintain a separation of clean materials from materials that have become contaminated during use.
  - 6 Provide a container(s) within the BSC for the collection of contaminated waste and other materials. Repeated movement out of the hood to discard pipettes or other waste materials can disrupt airflow in the cabinet and marginalize the protection to both the individual working at the BSC and to the cultures that are being manipulated.
    - a. Low profile, horizontal containers are preferable to vertical containers as they are less obstructive to airflow in the cabinet.
    - b. Contaminated items that will not be reused may be placed into small biohazard bag or a similar container.
    - c. If chemical disinfection will be used for the decontamination of reusable items an appropriate disinfectant should be poured into the discard container prior to use.
- Alternatively.
- d. If contaminated materials will be sterilized by autoclaving add enough water to the discard pan to ensure that sufficient steam is generated during autoclaving.
- 7 Do not work with open flames or other heat sources. These generate heated convection currents that may disrupt the smooth flow of air in the hood and may also damage the hood's HEPA filters.

#### ***Completion of work in the BSC***

- 1 Discard all waste materials generated by your work into appropriate containers inside the BSC. Close or cover all open containers.
- 2 Allow the cabinet to run for 3 to 5 minutes with no activity.
- 3 Disinfect the surfaces of all materials, equipment and containers that will be removed from the BSC, to minimize subsequent contamination in the laboratory.
- 4 Remove contaminated gloves and dispose of them appropriately
- 5 After putting on a clean pair of gloves, remove all materials from the BSC.
- 6 Wipe down all interior surfaces of the BSC with an appropriate disinfectant.
- 7 If the BSC is not scheduled for subsequent use, turn off the fluorescent light and cabinet blower. BSCs are designed for 24 hour operation, but in the interest of energy

conservation it should be shut down when it will not be used for an extended period of time.

- 8 Turn off the UV light if the cabinet is equipped and if appropriate.

### ***Spills in a Biological Safety Cabinet***

Leave the Biological Safety Cabinet turned on!

During the cleanup of spills, gloves should be changed whenever they become contaminated, after the work surface is decontaminated and before placing a new absorbent liner in the cabinet. Unless working with a double pair of gloves, hands should be washed whenever gloves are changed.

**Small Spills** that occur while working in the BSC should be handled immediately to avoid subsequent spread of the contaminating material.

1. Protect the other materials you are working with from possible contamination by closing culture plates, flasks, bottles of media, etc.
2. If the spill has been absorbed by a liner, carefully fold the liner over on itself so that further contact with spilled material is prevented by the liner's plastic backing. Otherwise contain the spill with absorbent materials and apply an appropriate disinfectant.
3. Any splatter onto items within the cabinet, as well as the cabinet interior, should be immediately cleaned with absorbent paper toweling soaked with disinfectant.
4. The materials used for cleanup should be put into a biohazard bag or wrapped in a leak-proof liner before removal from the BSC for proper disposal.
5. After decontamination is complete, allow the BSC to run for 3 to 5 minutes before resuming your work.

**Large Spills** that result in liquids flowing across the work surface or through the front or rear grills require more extensive decontamination.

1. Contain the spill with absorbent material
2. All items within the cabinet should be surface decontaminated and removed from the BSC.
3. Apply an appropriate decontaminating solution onto the work surface and if necessary through the grill(s) and into the drain pan or lower plenum and allow 20 to 30 minutes contact time for decontamination; this varies with the disinfectant and the infectious agent. Consult the MSDS for the agent and/or the manufacturer's directions for the disinfectant.
4. The spilled fluid and disinfectant solution on the work surface should be absorbed with paper towels and discarded into a biohazard bag before removal from the BSC.

5. If material was spilled through the cabinet's grills, the work surface must be removed after it is decontaminated to allow access and cleaning of the drain pan or lower plenum.
6. If the BSC is equipped with a drain valve, the spilled liquid and decontaminating solution may be emptied through a piece of flexible tubing attached to the drain valve and into a collection vessel containing additional disinfectant. The tubing should be of sufficient length to allow the open end to be submerged in the disinfectant within the collection vessel. For BSC's without a drain valve, the spilled liquid and disinfectant should be absorbed with paper towels and discarded into a biohazard bag.
7. Should the spilled liquid contain radioactive material, a similar procedure can be followed. Contact OSHS 906-487-2118 for specific instructions.

## **Routine Maintenance of a Biological Safety Cabinet**

### ***Weekly or as needed***

1. With the cabinet running verify that the reading on the pressure gauge is within the correct range for safe operation of the BSC.
2. Disinfect all surfaces inside the cabinet with 70% ethanol or another appropriate disinfectant. To facilitate cleaning a tool similar to the swiffer sweeper may be used to manipulate a disposable towel saturated with 70% ethanol into the less accessible regions of the cabinet.
3. The glass, front sash of the cabinet may also be cleaned with 70% ethanol or glass cleaner.
4. If UV light is used in conjunction with chemical disinfectants for the disinfection of interior cabinet surfaces, the light must be cleaned at least weekly with 70% ethanol to ensure that the light's effectiveness is not diminished by the accumulation of dust or other deposits on its surface.

### ***Monthly or as needed***

1. Clean the exterior surfaces of the BSC with a damp cloth or disposable towel. Pay particular attention to remove any accumulated dust on the front and top of the cabinet
2. Disinfect all surfaces inside the cabinet with 70% ethanol or another appropriate disinfectant. To facilitate cleaning a tool similar to the swiffer sweeper may be used to manipulate a disposable towel saturated with 70% ethanol into the less accessible regions of the cabinet.
3. Remove the cabinet work surface for access to the lower plenum / drain pan. Dirty drain pan surfaces and grilles, may eventually block the drain valve and/or obstruct airflow in the cabinet. Apply 70% ethanol to the dirty surfaces and wipe them carefully using disposable paper towels. Use caution while wiping these surfaces to avoid injury from broken glass that may be present and sharp metal edges. Never leave toweling on the

drain pan because the paper could block the drain valve or the air passages in the cabinet.

***Annually***

Have the biological safety cabinet certified by a qualified technician.

**Additional Information Regarding Biological Safety Cabinets and Their Use:**

[Biosafety in Microbiological and Biomedical Laboratories Appendix A](#)

[Proper use of a Biological Safety Cabinet](#) training video.

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## **BIOSAFETY LEVELS**

### **Biosafety Level 1 Practices and Facilities**

(From *Biosafety in Microbiological and Biomedical Laboratories*, BMBL 5<sup>th</sup> edition)

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

The following standard practices, safety equipment, and facility requirements apply to BSL-1.

#### ***Standard Microbiological Practices***

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.

5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:
  - a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
  - b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
  - c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
  - d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport.
  - a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
  - b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. The sign may include the name of the agent(s) in use, and the name and phone number of the laboratory supervisor or other responsible personnel. Agent information should be posted in accordance with the institutional policy.
10. An effective integrated pest management program is required. (See BMBL, Appendix G.)
11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an

individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

### ***Special Practices***

None required.

### ***Safety Equipment (Primary Barriers and Personal Protective Equipment)***

1. Special containment devices or equipment, such as BSCs, are not generally required.
2. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
3. Wear protective eyewear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses in laboratories should also wear eye protection.
4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Wash hands prior to leaving the laboratory. In addition, BSL-1 workers should:
  - a. Change gloves when contaminated, when glove integrity is compromised, or when otherwise necessary.
  - b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
  - c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

### ***Laboratory Facilities (Secondary Barriers)***

1. Laboratories should have doors for access control.
2. Laboratories must have a sink for hand washing.
3. The laboratory should be designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
  - a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

- b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
- 5. Laboratories windows that open to the exterior should be fitted with screens.

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## Biosafety Level 2 Practices and Facilities

(From Biosafety in Microbiological and Biomedical Laboratories, BMBL 5<sup>th</sup> edition)

**Biosafety Level 2** builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that: 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment. The following standard and special practices, safety equipment, and facility requirements apply to BSL-2.

### ***Standard Microbiological Practices***

- 1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
- 2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
- 3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
- 4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
- 5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:
  - a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
  - b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.

- c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
  - d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
  7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
  8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:
    - a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
    - b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
  9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include: the laboratory's biosafety level, the Laboratory supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the departmental policy.
  10. An effective integrated pest management program is required. (See BMBL, Appendix G.)
  11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

### ***Special Practices***

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.

2. Laboratory personnel must be provided medical surveillance, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory.
3. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.
4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.
6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
  - a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
  - b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
9. Animal and plants not associated with the work being performed must not be permitted in the laboratory.
10. All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.

***Safety Equipment (Primary Barriers and Personal Protective Equipment)***

1. Properly maintained BSCs, other appropriate personal protective equipment, or other physical containment devices must be used whenever:
  - a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.

- b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.
2. Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials. Remove protective clothing before leaving for non-laboratory areas, e.g., cafeteria, library, and administrative offices). Dispose of protective clothing appropriately, or deposit it for laundering by the institution. It is recommended that laboratory clothing not be taken home.
3. Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.
4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should:
  - a. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
  - b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
  - c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
5. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

#### ***Laboratory Facilities (Secondary Barriers)***

1. Laboratory doors should be self-closing and have locks in accordance with the institutional policies.
2. Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.
3. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

- a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
- b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
5. Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.
6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Vacuum lines should be protected with liquid disinfectant traps.
8. An eyewash station must be readily available.
9. There are no specific requirements for ventilation systems. 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.
10. HEPA filtered exhaust air from a Class II BSC can be safely recirculation back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly exhausted to the outside through a hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.
11. A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

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## Biosafety Level 3 Practices and Facilities

(From *Biosafety in Microbiological and Biomedical Laboratories*, BMBL 5<sup>th</sup> edition)

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures.

All procedures involving the manipulation of infectious materials must be conducted within BSCs or other physical containment devices.

A BSL-3 laboratory has special engineering and design features. The following standard and special safety practices, equipment, and facility requirements apply to BSL-3.

### ***Standard Microbiological Practices***

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.

Precautions, including those listed below, must always be taken with sharp items. These include:

- a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
- b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
- c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
- d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

Depending on where the decontamination will be performed, the following methods should be used prior to transport:

- a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
- b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
10. An effective integrated pest management program is required. See BMBL Appendix G.
11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

### ***Special Practices***

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
2. Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.
3. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.
4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents.
6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.

7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
  - a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
  - b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.
10. All procedures involving the manipulation of infectious materials must be conducted within a BSC, or other physical containment devices. No work with open vessels is conducted on the bench. When a procedure cannot be performed within a BSC, a combination of personal protective equipment and other containment devices, such as a centrifuge safety cup or sealed rotor must be used.

#### ***Safety Equipment (Primary Barriers and Personal Protective Equipment)***

1. All procedures involving the manipulation of infectious materials must be conducted within a BSC (preferably Class II or Class III), or other physical containment devices.
2. Workers in the laboratory where protective laboratory clothing with a solid-front, such as tie-back or wrap-around gowns, scrub suits, or coveralls. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when contaminated.
3. Eye and face protection (goggles, mask, face shield or other splash guard) is used for anticipated splashes or sprays of infectious or other hazardous materials. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories must also wear eye protection.
4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-3 laboratory workers:
  - a. Changes gloves when contaminated, glove integrity is compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.

- b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
  - c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
5. Eye, face, and respiratory protection must be used in rooms containing infected animals.

### **Laboratory Facilities (Secondary Barriers)**

- 1. Laboratory doors must be self-closing and have locks in accordance with the institutional policies. The laboratory must be separated from areas that are open to unrestricted traffic flow within the building. Laboratory access is restricted. Access to the laboratory is through two self-closing doors. A clothing change room (anteroom) may be included in the passageway between the two self-closing doors.
  - 2. Laboratories must have a sink for hand washing. The sink must be hands-free or automatically operated. It should be located near the exit door. If the laboratory is segregated into different laboratories, a sink must also be available for hand washing in each zone. Additional sinks may be required as determined by the risk assessment.
  - 3. The laboratory must be designed so that it can be easily cleaned and decontaminated. Carpets and rugs are not permitted. Seams, floors, walls, and ceiling surfaces should be sealed. Spaces around doors and ventilation openings should be capable of being sealed to facilitate space decontamination. Laboratory
    - a. Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Consideration should be given to the installation of seamless, sealed, resilient or poured floors, with integral cove bases.
    - b. Walls should be constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.
    - c. Ceilings should be constructed, sealed, and finished in the same general manner as walls.
- Decontamination of the entire laboratory should be considered when there has been gross contamination of the space, significant changes in laboratory usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the laboratory must be based on the risk assessment.
- 4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning.
    - a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

- b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
  - 5. All windows in the laboratory must be sealed.
  - 6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
  - 7. Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
  - 8. An eyewash station must be readily available in the laboratory.
  - 9. A ducted air ventilation system is required. This system must provide sustained directional airflow by drawing air into the laboratory from “clean” areas toward “potentially contaminated” areas. The laboratory shall be designed such that under failure conditions the airflow will not be reversed.
    - a. Laboratory personnel must be able to verify directional airflow. A visual monitoring device, which confirms directional airflow, must be provided at the laboratory entry. Audible alarms should be considered to notify personnel of air flow disruption.
    - b. The laboratory exhaust air must not re-circulate to any other area of the building.
    - c. The laboratory building exhaust air should be dispersed away from occupied areas and from building air intake locations or the exhaust air must be HEPA filtered.
- HEPA filter housings should have gas-tight isolation dampers, decontamination ports, and/or bag-in/bag-out (with appropriate decontamination procedures) capability. The HEPA filter housing should allow for leak testing of each filter and assembly. The filters and the housing should be certified at least annually.
- 10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer’s recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly exhausted to the outside through a hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be certified at least annually to assure correct performance. Class III BSCs must be directly (hard) connected up through the second exhaust HEPA filter of the cabinet. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.
  - 11. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, or other validated decontamination method).

12. Equipment that may produce infectious aerosols must be contained in primary barrier devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters should be tested and/or replaced at least annually.
13. Facility design consideration should be given to means of decontaminating large pieces of equipment before removal from the laboratory.
14. Enhanced environmental and personal protection may be required by the agent summary statement, risk assessment, or applicable local, state, or federal regulations. These laboratory enhancements may include, for example, one or more of the following: an anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities; gas tight dampers to facilitate laboratory isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; and advanced access control devices, such as biometrics.

The BSL-3 facility design, operational parameters, and procedures must be verified and documented prior to operation. Facilities must be re-verified and documented at least annually.

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## **DISPOSAL OF BIOLOGICAL AND MEDICAL WASTE**

Each laboratory is responsible for the proper collection and disposal of biological and medical waste. The regulated materials described below as well as any items contaminated by contact with these materials should be separated from the regular waste stream and collected in appropriate containers. Proper disposal of these regulated materials is detailed below.

### **Regulated Materials**

Michigan's medical waste regulatory act defines the rules governing the disposal of biological and medical waste. Regulated materials under this act include:

1. Cultures and stocks of infectious agents and associated biologicals, including laboratory waste, biological production wastes, discarded live and attenuated vaccines, culture dishes, and related devices.
2. Liquid human and animal waste, including blood and blood products and body fluids, but not including urine or materials stained with blood or body fluids.
3. Pathological waste such as human organs, tissues, body parts other than teeth, products of conception, and fluids removed by trauma or during surgery or autopsy or other medical procedure, and not fixed in formaldehyde.
4. Sharps including needles, syringes, scalpels, and intravenous tubing with needles attached.
5. Contaminated wastes from research animals that have been exposed to agents infectious to humans.

### ***Solid Waste***

Solid waste including culture plates, flasks and other disposable materials containing or contaminated with regulated waste materials such gloves, or materials used for cleaning/absorbing spills of blood or other biological liquids shall be collected in a durable leak proof container. This container shall be prominently labeled for biohazardous waste collection and equipped with a lid to minimize exposure to discarded waste material. A five gallon biohazard disposal bucket available for under \$25 from a variety of vendors is recommended. The carrying handle and smaller size of the 5 gallon bucket allow for easy and safe transport of biological waste from the laboratory to an autoclave for decontamination. Containers for collection of solid biological and medical waste should be lined with a clear, autoclavable, polypropylene bag. Do not collect regulated waste in bags that are printed with the biohazard symbol and/or labeled for biohazardous waste. Even after decontamination in an autoclave, these bags will not be accepted for final disposal in local sanitary landfills and will consequently require additional expense for disposal.

Do not overfill or compact waste in the container. When the container is approximately 3/4 full, loosely gather the top of the autoclavable liner into the bucket/container and close the lid for safe transport to an autoclave. At the autoclave, remove the bag from the bucket and place into an autoclavable secondary container such as a Nalgene tray. Add about 250ml of

water to the bag. This will facilitate the generation of steam within the bag necessary for decontamination of the waste. Before loading into the autoclave, place a chemical sterilization indicator into the bag so that it is located near the middle of the load, and then **loosely** secure the top of the bag with autoclave tape. **Do Not** seal the bag. For effective sterilization to occur it is critical that air and steam are able to move freely into and out of the bag during the autoclave cycle.

Depending on the size of the autoclave, multiple bags of waste may be decontaminated in a single autoclave run. However, do not overload the autoclave since decontamination is dependent upon the size of the load. If in doubt a smaller load is better. Typically, biological waste should be processed for a minimum of 60 minutes. After autoclaving, remove the chemical indicator from the bag and verify that conditions during the run were adequate for sterilization. Adequately processed waste may be discarded into the regular waste stream as non-hazardous waste.

#### **Liquid waste**

Blood and other body fluids may be disposed of without prior disinfection by pouring down the drain of an appropriate laboratory sink and into the sanitary sewer (A sink that is not used for hand-washing is preferred). Use a sufficient amount of water to rinse all the material into the drain. Following disposal, decontaminate the surfaces of the sink with an appropriate disinfectant such as a 10% solution of bleach and finally rinse with water to remove any residue.

Other liquid biological wastes generated in the laboratory such as spent culture media must be decontaminated either by chemical treatment or autoclaving prior to being discarded into the sanitary sewer. Chemical treatment is recommended since it allows for treatment of waste in the laboratory and thereby reduces the potential for spills or exposures during transport to the autoclave.

For chemical decontamination of liquid biological waste using bleach: add household bleach (~5% sodium hypochlorite) to the container of liquid waste so that the final volume of the solution is 10% bleach. Mix gently and allow a minimum of 30 minutes contact time before discarding into the sanitary sewer. Longer contact times may be required for some infectious agents. Contact the Biosafety Officer ([ibc@mtu.edu](mailto:ibc@mtu.edu)) for additional information.

Do not autoclave liquid wastes that have been treated with bleach. Bleach is very corrosive to the interior surfaces of an autoclave and reduces the useful life span of the autoclave and will void manufacturers' warranties.

For decontamination of liquid biological waste in the autoclave:

1. Verify that the container holding the liquid is able to withstand autoclave temperatures and is of adequate size to minimize the potential for boil-over in the autoclave. The container should be filled to no more than about 1/3 of its capacity.
2. Cover the container loosely with a lid or piece of aluminum foil, place it into an autoclavable secondary container and use a cart to transport the waste to an autoclave.

3. Autoclave the liquid biological waste using the liquid cycle/slow exhaust for a minimum of 60 minutes. A chemical indicator such as a diack sterilization monitor should be suspended in the liquid to verify that it has reached sterilization temperatures during the run.
4. Upon completion of the run, examine the chemical indicator to ensure that decontamination was successful before discarding the solution into the sanitary sewer.

### ***Pathological Waste***

Human pathological waste as defined by Michigan's medical waste act and similar waste generated from research with animals shall be handled in the same manner. Items for disposal shall be contained in a sealed plastic bag and clearly labeled as pathological waste. Pathological waste may be stored temporarily in the laboratory's refrigerator or freezer until arrangements can be made for proper disposal. Contact Jon Stone (Supervisor, Animal Care Facility) 7-2878, [jcstone@mtu.edu](mailto:jcstone@mtu.edu) to arrange for the transfer of pathological waste to the animal care facility and subsequent disposal by incineration.

### ***Sharps***

According to Michigan's Medical Waste Act, "Sharps means needles, syringes, scalpels, and intravenous tubing with needles attached." For purposes of collection and disposal at Michigan Tech, the definition of sharps may be expanded to include glass microscope slides, Pasteur pipettes, microtome blades, capillary tubes and any other items that are contaminated with infectious or potentially infectious biological materials and are capable of causing puncture wounds or lacerations if handled improperly. This working definition of sharps is not limited to those items that are specifically used in medical or biological procedures and with the exception sharps that are contaminated with hazardous, toxic or radioactive chemicals, includes sharps generated from all other uses.

Guidelines for the disposal of sharps:

1. Never discard needles or other disposable sharp instruments into the regular trash or into bags containing hazardous waste.
2. Immediately discard all used sharps directly into puncture-resistant containers that are specifically designed / designated for the collection of sharps.
3. Michigan's medical waste regulations require that containers holding discarded needles, syringes and/or scalpels are disposed of within 90 days of initial use. Selection of container size should be based upon the anticipated volume of sharps that will be generated during a 90 day period. Containers for sharps disposal can be purchased from a variety of vendors.
4. Sharps containers used for the collection of needles, syringes and/or scalpels shall be dated when they are put into use and shall not be kept in the laboratory beyond 90 days from their initial use.
5. Pasteur pipettes and other disposable glass items that are not contaminated with potentially infectious materials may be discarded into broken glass containers.

6. Disposable syringes with needles shall be discarded as a unit. If the needle must be removed, use the integrated device for needle removal found on most sharps containers or use other mechanical means.
7. Used needles and other contaminated sharps shall not be bent, broken, cut, recapped, re-sheathed or otherwise manipulated by hand. If any of the actions described above are required by a specific procedure, they will be done using a mechanical device such as pliers or hemostats.
8. Do not over-fill sharps containers. They should be closed and sealed when they are approximately 3/4 full.
9. If the outside of a filled sharps container is contaminated, the surface of the container should be chemically disinfected or the container autoclaved prior to being removed from the laboratory.
10. Sharps containers used for the collection of discarded needles, syringes and scalpels cannot be put into the trash. Even after decontamination, these sharps are considered as regulated medical waste and must be disposed of in accordance with regulations.
11. The final disposal of sharps containers is coordinated by the University. Filled sharps containers will be collected at a central location within the department/building. Pick-up for final disposal will be on a quarterly basis to coincide with the state mandated 90 day limit for the storage of used sharps.

For additional information regarding the disposal of biological/medical waste or to arrange for pickup and subsequent disposal of filled sharps containers, contact the Biosafety officer 7-2902, (Office of Research Integrity and Compliance) or Occupational Safety and Health Services 7-2118

## **INCIDENT RESPONSE**

### **Biological Spills**

The consequences of a spill may be minimized by covering the laboratory bench or work surface with a plastic baked absorbent liner, when working with hazardous or potentially hazardous organisms or biological materials,

A spill kit containing the following items must be available in the laboratory:

1. an appropriate disinfectant solution (such as 10% dilution of household bleach),
2. a package of paper towels,
3. gloves,
4. autoclave bags,
5. sharps container, and
6. forceps to pick up broken glass.

#### ***Preventing and minimizing personal exposure takes priority.***

If a biological spill is beyond your capacity to safely clean up:

1. Notify others working in the laboratory and evacuate immediately.
2. Close the laboratory door to restrict access to the spill area.
3. If you or other individuals are exposed, immediately remove contaminated protective equipment and clothing and wash affected areas with soap and water and rinse for up to 15 minutes.
4. Seek assistance (Public safety 911 or 487-2216; Biosafety Officer, 487-2131; Occupational Safety and Health Services 487-2118)
5. If medical follow-up is warranted, it should be sought immediately.

#### ***Spill Cleanup (General Guidelines - BSL-1 Material)***

1. Wear gloves and lab coat.
2. Use forceps to pick up broken glass and discard into SHARPS container.
3. Cover spilled material with absorbent paper towels or other absorbent material.
4. Add diluted disinfectant in sufficient quantity to ensure effective microbial inactivation.
5. Dispose of towels in biohazard waste container.
6. Wipe spill area with diluted disinfectant.
7. Discard all contaminated materials into biohazard waste container

8. Wash hands with soap and water when finished.

***Spill of BSL-2 Material***

1. Keep other workers out of the area to prevent spreading spilled material. Post warning sign, if needed.
2. Remove contaminated clothing and put into a biohazard bag for decontamination.
3. Wash hands and exposed skin and inform the laboratory supervisor of the spill. Call the Biological Safety Officer at 7-2131 for assistance, if necessary.
4. Put on protective clothing (lab coat, gloves and if needed, face protection and shoe covers) and assemble clean-up materials (disinfectant, autoclavable container or bag, forceps, sharps container, and paper towels).
5. Pick up broken glass with forceps and dispose into SHARPS container.
6. Cover the spill with paper towels and add appropriately diluted disinfectant.
7. After at least 20 minutes contact time, pick up the paper towels and re-wipe the spill area with diluted disinfectant.
8. Collect all contaminated materials into a biohazard waste container and sterilize in an autoclave.
9. Wash hands with soap and water.

***Spill of BSL-3 Material***

- 1 Stop work immediately.
- 2 Avoid inhaling airborne material while quickly leaving the room. Notify others to leave. Close door, and post with warning sign.
- 3 Remove contaminated clothing, turn exposed areas of clothing inward, and place in a biohazard bag. Wash hands with soap and water.
- 4 Notify the laboratory supervisor. Call the Biological Safety Officer at 487-2131 for assistance, if necessary.
- 5 Allow 60 minutes for aerosols to disperse before re-entering the laboratory to begin clean-up.
- 6 Put on personal protective equipment (N95 or other HEPA filtered respirator, gown, gloves, and shoe covers) and assemble clean-up materials (disinfectant, autoclavable container or bag, forceps, sharps container, and paper towels).
- 7 Contain the spill with absorbent paper towels or disposable pads. Carefully soak the spill with a 1:10 dilution of household bleach (or other disinfectants which are effective on the suspected biological agent); avoid creating aerosols when pouring the disinfectant. Leave the room and allow 30 minutes for the disinfectant to inactivate the material.

- 8 Pick up broken glass with forceps and discard in SHARPS container.
- 9 Clean up liquid with paper towels and collect all contaminated materials into biohazard bag or container. Remove all spilled materials and decontaminate the area again with an appropriate disinfectant.
- 10 Autoclave (or soak in 1:10 dilution of household bleach) lab coat, gloves, and other protective equipment that were worn for clean up.
- 11 Wash hands thoroughly with soap and water.

#### ***Spill of Human Blood***

- 1 Wear gloves and lab coat to clean up spill.
- 2 If broken glass is present, use forceps to pick up and place in SHARPS container.
- 3 Absorb blood with paper towels and discard in biohazard waste container.
- 4 Using a dilute bleach solution, clean the spill site of all visible blood.
- 5 Wipe the spill site with paper towels soaked in a disinfectant such as household bleach diluted 1:10.
- 6 Discard all contaminated materials into biohazard waste container.
- 7 Wash hands with soap and water.

## **Injury Involving Biological Materials**

#### ***Severe Injuries***

- 1 Call 911 for assistance and transportation to Portage Health. If the injured person is also potentially contaminated with a harmful chemical or biological material give this information to the dispatcher so that emergency responders can arrive prepared to deal with the additional hazards.
- 2 Give first aid if trained to do so and only if you can do so without exposing yourself to danger
- 3 A designated member of the laboratory will provide information about the accident/exposure and if an exposure is involved provide a copy of the Material Safety Data Sheets to emergency responders and to medical staff at the hospital.
- 4 Report incident to the Principal Investigator and to the University Occupational Safety and Health and Services [report form](#).

#### ***Contamination to the Body***

- 1 Immediately remove contaminated clothing and drench skin with water. Wash with soap and water, and flush the area for 15 minutes. Avoid the use of bleach and other disinfecting agents that are caustic to the skin.

2. Call 911 for assistance and transportation to Portage Health. Tell the dispatcher that the individual is potentially contaminated with a harmful chemical or biological material so that emergency responders can arrive prepared to deal with the additional hazard.
3. Report the incident to the laboratory supervisor/Principal Investigator and to the University Occupational Safety and Health and Services [report form](#).

#### ***Splash to the Eye***

1. Immediately flush the eye with a gentle stream of clean, temperate water for 15 minutes. Hold the eyelid open. Be careful not to wash the contaminant into the other eye.
2. Seek additional medical assistance from first aid providers, if necessary.
3. Call 911 for assistance and transportation to Portage Health.
4. Report the incident to the laboratory supervisor/Principal Investigator and to the University Occupational Safety and Health and Services [report form](#).

#### ***Punctures/Laceration of the Skin***

1. Wash the affected area thoroughly with soap and water, and rinse the area for up to 15 minutes.
2. Allow small wounds such as punctures to bleed freely. There is no evidence that additional squeezing or “milking” of the puncture site is beneficial in preventing infection.
3. Control more severe bleeding with direct compression on the wound with sterile dressings. Wear latex/rubber gloves to prevent exposure to biohazards/Blood/Body fluids.
4. The application of antiseptics or disinfectants may be beneficial, however, avoid the use of bleach and other disinfecting agents that are caustic to the skin.
5. If necessary call 911 for assistance and transportation to Portage Health.
6. Report the incident to the laboratory supervisor/Principal Investigator and to the University Occupational Safety and Health and Services [report form](#).

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## **PERSONAL PROTECTIVE EQUIPMENT (PPE)**

Personal protective equipment (PPE) includes all clothing and other accessories worn as a barrier against laboratory hazards. Examples of PPE include laboratory coats or gowns, gloves, safety glasses, face shields, masks and respirators. The appropriate use of PPE is only one of a variety of different strategies used to increase personal safety in the laboratory. It must be recognized that the use of PPE does not eliminate the hazard nor can it compensate for poor technique or disregard for established safety practices. However, when PPE is used correctly and as a supplement to the diligent use of good laboratory techniques and practices, it can further minimize the risk of personal exposure to hazardous materials in the laboratory.

Where there is a risk of personal exposure to hazardous materials, primary barriers such as a chemical fume hood, a biosafety cabinet, a plexiglas splash guard or other engineering controls are used as the first level of protection. When these primary barriers are not sufficient to prevent personal exposure, they should be supplemented with appropriate PPE. In some situations where it is impractical to use a biosafety cabinet or other engineering controls, personal protective equipment may form the primary barrier between an individual and hazardous or infectious materials.

As with all other engineering and work practice controls the selection and use of PPE should be based upon a risk assessment of the hazards associated with the specific laboratory procedure that is being performed. For example gloves should be worn when it can be reasonably expected that hands may come into contact with hazardous materials. Similarly, a face shield/mask should be worn to protect the face, eyes, nose, and mouth, if a procedure that may generate a splash cannot be performed safely behind an engineered splash guard. In many cases published recommendations or standard operating procedures can serve as a guide for selecting appropriate PPE. Contact [ibc@mtu.edu](mailto:ibc@mtu.edu) for additional information.

While protection of individuals working in the laboratory is the foremost consideration for the use of PPE, protecting experiments from contamination by the worker may also be used as justification for the use of PPE. This may include the use of PPE for invasive/surgical procedures, sensitive bioassays or PCR experiments.

Regardless of the reason for wearing PPE, it should be removed when experiments are completed and should never be worn outside the laboratory.

Finally, it should be noted that inappropriate use of PPE defeats its intended purpose and may increase risk. For example, failing to remove contaminated gloves before touching clean surfaces may expose others in the laboratory to risk; wearing a lab coat outside the laboratory may spread contamination to public areas; or using a respirator designed for use with chemical solvents will not protect the wearer from hazardous particulates.

## **LABORATORY COATS**

Laboratory coats are available in a variety of different materials and fabrics and are principally worn to protect clothing and arms from inadvertent contamination while working in the laboratory. It is important to choose a coat based on the level of protection that is needed. For example a lab coat worn to protect against spilled or splashed liquids should be capable of preventing liquids from soaking through and contaminating an individual's clothing or skin. If there are specific concerns about a splash to the front of the coat, a solid front gown or an apron worn in conjunction with a laboratory coat may be a more appropriate choice.

Similarly, Many laboratory coats, particularly disposable coats made from synthetic material may offer very little protection if they come into contact with a heat source. If exposure to open flames or other heat sources is a concern, laboratory coats designated as fire resistant (FR) should be considered.

Laboratory coats with knit or closed cuffs are preferred since they reduce the possibility of hanging cuffs coming into contact with hazardous materials and can offer more protection for the wrist and lower arm. For critical work where it is essential to prevent exposures to the lower arm, gloves can be easily pulled over the sleeve of a lab coat with a knit cuff.

Laboratory coats should be discarded or laundered when they become soiled or contaminated. Laboratory coats that are grossly contaminated with infectious material should be disinfected immediately with either a chemical disinfectant or by autoclaving before they are laundered or disposed of as decontaminated waste. Disposable lab coats are recommended in situations where there are no departmental or laboratory facilities for laundering. Lab coats should not be taken home for laundering.

## **GLOVES**

There is no single glove type that is suitable for all applications. Like all other PPE, gloves should be selected and used based upon the hazards and specific requirements of the procedure being performed. For example, delicate work requires the use of thin, flexible gloves (alternatives to latex gloves should be available); chemically resistant gloves are required when handling solvents; and thermally resistant gloves are needed when operating autoclaves or storing/retrieving materials at ultra-low temperatures.

In addition to the general hazards that may be encountered in any laboratory, biology and microbiology laboratories may have specific hazards associated with infectious agents or other biological materials that require the use of gloves. These hazards include but are not limited to bacterial, viral or fungal pathogens classified as risk group 2/BSL-2 (or greater) or toxins derived from biological sources. Gloves are also specifically recommended for recombinant DNA work involving organisms from risk group 2/ BSL-2 (or greater) or DNA sequences derived from these organisms. For all other work involving recombinant DNA, gloves may be worn as a method of protecting experiments from personal and environmental contamination.

Additionally, the OSHA Blood borne Pathogen Standard requires the use of gloves when it can be reasonably expected that hands may come into contact with blood or blood products and otherwise potentially infectious material such as human cell lines.

Gloves are not specifically required when working with Risk Group 1/BSL-1 organisms, defined as well characterized agents that are not associated with disease in healthy adult humans and present minimal potential risk to laboratory personnel and the environment. However, Glove selection and use should always be based on an appropriate risk assessment. For example, gloves are required for work with these organisms if 1) the skin on the hands is broken, dry or cracked or if a rash is present; 2) hazardous materials (chemical, radioactive, etc.) are used in conjunction with the risk group 1 agent; or 3) an individual has a condition that may increase their susceptibility to infection. (See Guidance for Completing the Laboratory Risk Assessment, Question 9);

**The following apply to the use of gloves as PPE in the laboratory.**

1. Select and use gloves that are appropriate for the procedure.
2. Never touch your eyes, nose, mouth or your face while wearing gloves.
3. To prevent the spread of contamination gloves must be removed before touching "clean" surfaces such as door knobs, computer keyboards, books, telephones/cell phones, etc. This rule applies to gloves worn to protect the user as well as gloves worn to protect the experiment.
4. Do not wash or reuse disposable gloves.
5. Dispose of single use gloves properly. For example, gloves that are contaminated with infectious agents or otherwise potentially infectious materials should be discarded as biological waste and autoclaved before their ultimate disposal as decontaminated medical/biological waste. Gloves contaminated with other materials (chemical, radioactive, etc.,) should also be disposed of according to the risk.
6. Gloves must be changed when they become contaminated, or if they are torn, punctured or otherwise compromised.
7. For critical experiments such as polymerase chain reaction (PCR) experiments, gloves should be changed often to avoid cross contamination between samples.
8. Gloves must be removed in a manner that prevents the unintentional transfer of hazardous or infectious material from the outside contaminated surfaces of the glove to unprotected skin or clothing
9. Consider wearing two pair of gloves for particularly hazardous work as this permits removal of a contaminated outer glove with minimal risk of exposure.
10. Gloves must not be worn outside the laboratory. If a hazardous material or sensitive experiment needs to be transported to another laboratory, it should be placed into a secondary container that can be safely handled without gloves.

- Gloves must be removed and hands washed when tasks are completed and before leaving the laboratory. The importance of proper gloving techniques and frequent and thorough hand washing is emphasized. Care should be taken when manipulating faucet handles to prevent contamination of cleaned hands.

The sites listed below may be useful in selecting appropriate gloves for use in the laboratory. With regard to the chemical compatibility of gloves, the rating systems used by manufacturers vary. For example, a color code where red = bad, yellow = not recommended, green = good; or a letter code where E = excellent, G = Good, P = poor, NR = Not Recommended or any combination or variation on these schemes may be used. Please take time to understand the rating system before making a decision on the glove to be used. Note: Kimberly-Clark manufactures Fisherbrand purple nitrile and safeskin gloves and consequently these share the same link.

		
		
		

## EYE AND FACE PROTECTION

Eye and face protection such as safety glasses, goggles, face shield or similar PPE are worn when it can be reasonably expected that a splash or spray of infectious or other hazardous material may occur. Most importantly, all procedures, especially those requiring the use of hazardous materials, should be conducted in a manner that minimizes the creation of splashes, sprays or aerosols. These procedures may include but are not limited to pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, filtration using vacuum/pressure or opening containers of infectious or hazardous materials. Where there is a risk for a splash or spray, primary barriers such as a chemical fume hood, biosafety cabinet, plexiglas splash guard or other engineering controls are used as the first level of protection. When these primary barriers are not sufficient to prevent personal exposure, they should be supplemented with PPE specifically designed to protect the eyes and face.

The following apply to the use of eye and face protection in the laboratory:

- Select eye and face protection that is appropriate to the task being performed.
- Do not put on or remove face/eye protection while wearing gloves that are potentially contaminated.

3. Individuals who wear contact lenses in the laboratory should also wear eye protection. Contact lenses do not provide protection to the eyes. Foreign material splashed into the eye may become trapped under the contact lens and result in more serious injury.
4. If eye protection is deemed necessary in a laboratory, then an emergency eyewash station should also be available.
5. Eye and face protection must be decontaminated and cleaned before reuse or disposed of with other contaminated laboratory waste.
6. When hazardous or infectious materials must be handled outside of a biosafety cabinet, or other containment device, eye and face protection must be worn if there is a possibility that the procedure may create a splash or spray of harmful material.

## MASKS AND RESPIRATORS

The following information is given as guidance for the selection masks and respirators for use in the laboratory. As with other PPE, their use in the laboratory is based upon a risk assessment of the hazards associated with the specific procedure(s) being performed.

### *Surgical Masks*

Surgical masks do not provide respiratory protection against harmful/infectious aerosols, smoke or chemical fumes. Although they may be used to protect the mucous membranes of the mouth and nose from a splash or spray of hazardous liquid, other alternatives such as an engineered splash guard acting as a primary barrier or a face shield worn as PPE will provide superior protection in most situations.

### *Respirators*

Respirator use must be approved and administered through Michigan Tech's office of Occupational Safety Health Services respiratory protection program (for additional information contact OSHS at 487-2118 or [IBC@mtu.edu](mailto:IBC@mtu.edu)).

While respirators can reduce the risk of exposure to inhalational hazards, their use should be considered only as a last resort or a temporary control measure. In most laboratory situations a biological safety cabinet, a chemical fume hood or a similar engineering control will offer more effective protection against exposure. Accordingly, engineering controls, as well as administrative and work practice controls (written policies, rules, standard operating procedures, supervision, training, etc) should be put into place before respirator use is taken into consideration.

There are very few specific recommendations for the use of respirators to control or limit exposure to biological aerosols. Traditionally, recommendations for selection and use of a respirator are based upon the expected airborne concentration of the hazardous material and its occupational exposure limit that defines the level of exposure that may occur without serious adverse health effects. However, there are few methods for accurately measuring the airborne concentration of biological organisms in laboratory air and there is very little information on the safe exposure level to infectious or toxic biological materials.

Consequently all requests for respirator use to control exposure to hazardous biological aerosols are reviewed on case by case basis by the Director of Occupational Safety and Health Services (OSHS), the Biosafety Officer and the Institutional Biosafety Committee (IBC). Recommendations for respirator use will be based on a laboratory risk assessment, best available practices, current knowledge and professional judgment.

## References

- Wilson, D.E. and Chosewood, L.C., (eds.) 2009., *Biosafety in Microbiological and Biomedical Laboratories* 5<sup>th</sup> ed., Centers for Disease Control and Prevention, and National Institutes of Health., Washington, D.C., US Government Printing Office,
- Fleming, D.O. and Hunt, D.L. (eds) 2006., *Biological Safety: Principles and Practices* 4<sup>th</sup> ed., Washington, D.C., ASM Press.