



UNIVERSITY OF
MICHIGAN

U-M Biosafety Manual

THE MANDATORY SUPPLEMENTS TO THE UNIVERSITY OF MICHIGAN U-M BIOSAFETY MANUAL ARE
FOUND IN [SECTION 14](#)

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SECTION 1: INTRODUCTION

The University of Michigan (U-M) Biosafety Manual is a resource for information, guidelines, policies, and procedures that will encourage safe research and eliminate, or reduce, the potential for exposure to biohazards. The information presented here also reflects the requirements and guidelines of federal and state regulations.

The U-M Biosafety Manual is applicable to all laboratory, research, teaching, and support activities that may involve biohazards. Biohazards are microorganisms, microbial toxins, or other biological agents that can infect or cause disease in humans, animals, or plants. Biohazards may include bacteria, biological toxins, viruses, fungi, rickettsia, prions, protozoans, parasites, genetically modified organisms, and recombinant or synthetic nucleic acid molecules. In addition, biohazards include human blood, body fluid, tissues, and cell lines of human origin and certain animal-derived tissues, fluids, and cells.

The most current version of the U-M Biosafety Manual will be maintained on the Environment, Health & Safety (EHS) website. The U-M Biosafety Manual will be reviewed and updated annually by the U-M Biosafety Officer.

The U-M Biosafety Manual should not be considered the only reference to address biological safety. The Principal Investigator (PI) or supervisory personnel **must** complete lab-specific biosafety binder documents available on the EHS website (Biosafety Manual Tab) to provide instruction and guidance regarding specific practices and procedures conducted in their lab.

A U-M [Exposure Control Plan](#) (ECP) applies to all U-M departments whose employees may reasonably anticipate contact with blood or other potentially infectious materials (OPIM) during the performance of their duties. In compliance with the Michigan Occupational Safety and Health Administration (MIOSHA) Bloodborne Pathogens Standard, U-M requires all departments that fall within the scope of this policy to minimize employee risk from exposure and infection by implementing the U-M ECP.

Acknowledgement

PIs and all laboratory personnel active in research within laboratories under their charge **must** agree to comply with the provisions of the U-M Biosafety Manual and to complete the lab-specific biosafety documents to address hazard conditions which are specific to the laboratory spaces under their charge. The contents of this manual **must** be reviewed with laboratory personnel, and they **must** be given the opportunity to ask questions or voice concerns regarding their job description and work environment.

U-M Biological Materials Policy Statement

All research with potentially hazardous biological materials **must** be registered with the U-M [Institutional Biosafety Committee \(IBC\)](#). The potentially hazardous biological materials are as follows:

- Recombinant DNA and synthetic nucleic acid molecules (this includes [human gene transfer studies](#))
- Infectious agents
- Biological toxins
- Human-derived tissues, fluids, cells
- Certain animal-derived tissues, fluids, cells
- Federally-regulated Select Agents, experiments with Dual Use Research of Concern potential, and research requiring BSL3 containment

SECTION 2: RISK GROUP CLASSIFICATION

Risk groups are a method used by the World Health Organization (WHO) and by the National Institutes of Health (NIH) to classify human infectious agents based on the hazard to both the individual and to the community. There are four risk groups which are classified according to their relative pathogenicity for healthy adult humans.

RISK GROUP	DESCRIPTION	PORTAL OF ENTRY/TRANSMISSION	RISK ASSESSMENT	
Risk Group 1 (RG1)	Agents are defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans or animals.	Laboratory personnel may become infected through high doses or unusual routes of exposure that are not commonly encountered in a natural setting.	Opportunistic RG1 pathogens may cause serious disease in elderly persons, infants, and persons with compromised immune systems.	A risk assessment should be used for vaccine strains, as multiple passages in vivo do not ensure avirulence.
Risk Group 2 (RG2)	Agents are associated with a human or animal disease which is rarely serious and for which preventive or therapeutic interventions are often available.	RG2 organisms have the capability to cause serious disease based on dose, route of exposure, and immune status.	Laboratory exposures may cause serious infection, but the risk of spread of infection is limited.	The risk assessment should give special attention to those RG2 organisms for which preventative or therapeutic interventions are not available.
Risk Group 3 (RG3)	Agents are associated with a serious or lethal human or animal disease and have the potential for respiratory transmission, and for which preventive or therapeutic interventions may be available.			The risk assessment should give special attention to those RG3 organisms for which preventative or therapeutic interventions may not be available, or are less available than for RG2 organisms.
Risk Group 4 (RG4)	Agents are associated with serious or lethal human or animal disease, and for which there is no available vaccine or therapy.	RG4 agents may be transmitted via the aerosol route, can be readily transmitted from one individual to another, directly or indirectly.		Not Applicable: RG4 agents are not permitted at U-M.

SECTION 3: BIOSAFETY LABORATORY PRACTICES

The standard microbiological practices listed below apply to all biosafety containment levels. The [U-M Biological Safety Designated Standards](#) outlines the practices and facility requirements.

Standard Microbiological Practices

- Persons working in the laboratory **must** be fully aware of the potential hazards to themselves and their co-workers. Personnel **must** meet the specific entry/exit requirements for this space.
- Persons **must** wash their hands after working with potentially infectious materials and before leaving the laboratory.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in the laboratory
- Food **must** be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
- Mouth pipetting is strictly prohibited; mechanical pipetting devices **must** be used.
- 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.
- Laboratory coats, gloves, and protective eyewear **must** be worn when working in the laboratory.
- Non-experimental animals and plants are not permitted in the laboratory.

SECTION 4: BIOSAFETY LEVELS (BSL)

The following table identifies the agents used, facility requirements, and work practices for each biosafety level:

BIOSAFETY LEVEL	FACILITY REQUIREMENTS	STANDARD MICROBIOLOGICAL PRACTICES
BSL1 Agent(s) used: Noninfectious	<ul style="list-style-type: none"> Doors for access control Screens on windows that open to the exterior Non-fabric chairs and furniture easily cleanable Sink required <p>Special containment equipment or facility design is neither required nor generally used.</p>	<ul style="list-style-type: none"> Hands must be washed after working and before leaving lab NO food or drink in lab Safe sharps handling procedures Perform all procedures to minimize splashes and aerosols Decontaminate infectious material before disposal Signage to convey hazards within lab Personal protective equipment must be worn
BSL2 Agent(s) used: Infectious spread via blood or oral/fecal transmission Includes human blood, human cell lines, toxins, venom, materials from Nonhuman primates	<ul style="list-style-type: none"> All BSL1 requirements plus the following: Door(s) should be self-closing and lockable Vacuum lines protected Autoclave available or approved alternative decontamination method Laboratories should be under negative or neutral pressure Eyewash must be available 	All BSL1 requirements plus the following: <ul style="list-style-type: none"> Limited access Work must be conducted with door(s) closed Aerosol generating procedures must be conducted in a biosafety cabinet (BSC) Sharps precautions Lab-specific biosafety manual Laboratory personnel demonstrate proficiency (training must be documented) Medical surveillance as appropriate Offered available immunizations
BSL3 Agent(s) used: Infectious spread via aerosol transmission	All BSL1 and BSL2 requirements plus the following: <ul style="list-style-type: none"> Double door entry Negative air flow Biosafety cabinet(s) Hands free sink Laboratory must be sealable 	All BSL1 and BSL2 requirements plus the following: <ul style="list-style-type: none"> Controlled access All work conducted in biosafety cabinet
BSL4	No work at a BSL4 is conducted at the University of Michigan	

Animal Biosafety Levels

A similar set of four biosafety levels are provided for work with vertebrate animals infected with agents, which may infect humans. These Animal Biosafety Levels, ABSL 1 thru 4, outlines practices, equipment, and facilities that are comparable to the laboratory biosafety levels described previously.

BIOSAFETY LEVEL	FACILITY REQUIREMENTS	STANDARD MICROBIOLOGICAL PRACTICES
A-BSL1 Agent(s) used: Noninfectious	<ul style="list-style-type: none"> Doors for access control Screens on windows that open to the exterior Non fabric chairs and furniture easily cleanable Sink available for handwashing Emergency eyewash and shower readily available Special containment equipment or facility design is neither required nor generally used. 	<ul style="list-style-type: none"> Hands must be washed after working and before leaving lab NO food or drink in lab Safe sharps handling procedures Perform all procedures to minimize splashes and aerosols Decontaminate infectious material before disposal Signage to convey hazards within lab Personal protective equipment must be worn
A-BSL2 Agent(s) used: Infectious spread via blood or oral/fecal transmission Includes human blood, human cell lines, toxins, venom, materials from Nonhuman primates	All A-BSL1 requirements plus the following: <ul style="list-style-type: none"> Door(s) should be self-closing and lockable Vacuum lines protected Autoclave available or approved alternative decontamination method Laboratories should be under negative or neutral pressure Eyewash must be available 	All A-BSL1 requirements plus the following: <ul style="list-style-type: none"> Limited access Work must be conducted with door(s) closed Aerosol generating procedures must be conducted in a biosafety cabinet Sharps precautions Lab-biosafety manual Laboratory personnel demonstrate proficiency (training must be documented) Medical surveillance as appropriate Immunizations offered as available
A-BSL3 Agent(s) used: Infectious spread via aerosol transmission	All A-BSL1 and A-BSL2 requirements plus the following: <ul style="list-style-type: none"> Double door entry Negative air flow Biosafety cabinet(s) Hands free sink Laboratory must be sealable 	All A-BSL1 and A-BSL2 requirements plus the following: <ul style="list-style-type: none"> Controlled access All work conducted in biosafety cabinet
A-BSL4	No work at ABSL4 is conducted at the University of Michigan	

SECTION 5: LABORATORY ACCESS

Admittance

- The PI or his/her designee authorizes admission to the laboratory. Persons requesting to use the laboratory or equipment shall be advised of the potential hazards involved and shall follow all U-M biosafety requirements.
- Access to the laboratory is restricted when work with biohazardous materials is in progress, after hours, or when laboratory personnel are not available.

Security

Biohazardous organisms and toxins may be of interest to persons or groups involved in terrorism or other illegal activities. These materials could pose a serious threat to humans, agriculture, or the livestock industry and **must** be kept secured.

Vaccinations (if applicable)

Laboratory personnel **must** be provided with information regarding vaccines that may be available to protect them against laboratory-acquired infection. The PI or supervisory personnel may require immunization as a condition of employment. Vaccination information that should be provided to laboratory personnel includes: efficacy, side effects, booster schedule, etc. Vaccinations should be provided to laboratory personnel free of charge and during working hours. Vaccinations are provided through U-M Occupational Health Services (OHS). Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel should be provided with information regarding immune competence and conditions that may predispose them to infection.

Restrictions or Recommendations

Restrictions or recommendations will be made on an individual basis for entry or working within the lab. Examples of medical conditions that might warrant special precautions are HIV infection, immunosuppressive conditions, and drug therapies that suppress the immune system. Additionally, it is recognized that exposure to certain infectious agents may adversely affect a fetus during pregnancy if the mother is infected with the agent. Laboratory personnel, who fall into any of the above conditions, should inform their personal physician and the PI about the situation.

Biosafety Training

It is the direct responsibility of the PI or supervisory personnel to initially instruct new laboratory personnel of the safety procedures used in the laboratory. The PI is responsible for ensuring all laboratory personnel exhibit competency in good microbiological techniques prior to initiating experiments. This includes a thorough review of the appropriate operating procedures of the laboratory.

- Laboratory personnel are required to complete [EHS-Biosafety & Bloodborne Pathogen training](#). Training is available online through ([BLS101w](#)) or in person ([BLS101](#)). Additional EHS training may be required based on research focus.

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- U-M laboratory personnel who may reasonably anticipate contact with human blood, blood products, tissues, fluids or OPIM, including human cell lines during the performance of their duties **must** complete an annual [EHS-Biosafety & Bloodborne Pathogen training](#). Training is available online through (BLS101w) refresher training course.
 - Once a year all personnel working in the laboratory will be instructed by the PI or supervisory personnel about the special biological safety procedures to be used. Attendance at this session is mandatory and **must** be documented.
 - Laboratory personnel **must** have prior experience with the agent in use or **must** be provided with suitable and sufficient information, instruction, and training about working with the agent prior to initiating work. A training course entitled "[Working Safely with Viral Vectors](#)" is available for new or entry-level laboratory personnel who plan to use viral vectors in vitro or in vivo, and who cannot demonstrate significant previous experience and expertise in the necessary aspects of biosafety and regulatory compliance. The course is provided by the U-M Vector Core Laboratory and is recommended by the IBC and EHS. Registration for the course ([BLS008](#)) is available on the EHS website
 - New laboratory personnel **must** review the U-M Biosafety Manual and associated documents listed in the Biosafety Proficiency Training Form prior to starting work in the laboratory.

SECTION 6: CONTROLS TO REDUCE EXPOSURES

U-M Standards for Biological Laboratories

[U-M Biological Safety Designated Standards](#)

Negative Pressure Tissue Culture Rooms

In general, a separate tissue culture room provides a higher level of containment for working with biohazardous materials. Mechanical ventilation should provide an inward flow of air (negative pressure) without recirculation to spaces beyond the laboratory. U-M, EHS will verify air pressurization during inspections.

Bench Tops

- Bench tops should be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
- The work areas should be kept clean and dust free as to prevent contamination of samples and laboratory-acquired infections.

Laboratory Furniture

Laboratory furniture should be capable of withstanding anticipated loading and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.

Workplace Practice Controls

All personnel **must** routinely use appropriate controls when handling biohazards or materials that may harbor biohazards. These include:

Engineering Controls

- Primary Barriers (e.g., biological safety cabinets and their respective ventilation systems) are the preferable method for containment of biohazards. Aerosol-generating procedures with biohazardous materials that pose an inhalation risk should always be handled in a biosafety cabinet. A biosafety cabinet is designed to contain microorganisms, which are released during work within the cabinet. Selection of the type of primary barrier should be based on the risk assessment.
- Secondary Barriers (e.g., building design features include floor to ceiling walls, operating areas under negative pressure and using closed doors). Many laboratories have monitoring systems built-in to indicate system failures that could affect secondary containment. Laboratory personnel should be familiar with these devices if they are available.
- Sink is required for immediate handwashing.
- Emergency eyewash **must** be readily available.

Personal Protective Equipment

Personal Protective Equipment (PPE) should be selected in accordance with the hazards identified. The *minimum level of PPE* when working in biological laboratories should include **lab coats, safety glasses and appropriate gloves**. Alternatives to latex gloves should be available. Lab coats should not be taken home for laundering. Professional laundering service is available. (See [PPE Hazard Assessment in Chemical Hygiene Plan \(CHP\) appendix 1](#))

Additional PPE

PPE	DESCRIPTION
Hair Bonnet	May be required in certain animal laboratory spaces refer to PPE indicated on door sign.
Face Mask	May be required in non-human primate facilities for specific tasks.
N95 Respirator	May be required for certain allergies. Risk assessment required for use of this respiratory protection and entry into the EHS Respiratory Protection Program.
Face shield	May be required if there is a splash hazard that cannot be mitigated through other engineering controls. (See CHP appendix 1 NOTE 3 for proper use).
Double gloves	Two pairs of gloves may be required to mitigate risks for hazards such as needle sticks; to reduce the likelihood of cross contamination from handling biohazardous organisms; and for spill clean-up as determined by risk assessment.
Disposable sleeves	May be required as a supplement to lab coat or gown to mitigate risk of cross contamination.
Foot covers	May be required in certain animal laboratory spaces, refer to PPE indicated on door sign, to reduce the likelihood of cross contamination and for spill clean-up as determined by risk assessment.

PPE	DESCRIPTION
Scrubs	May be required in certain laboratory spaces, refer to PPE indicated on door sign or lab SOP, to reduce the likelihood of cross contamination and for spill clean-up as determined by risk assessment. May also be used as alternative protective clothing.
Tyvek suites	May be required in certain animal laboratory spaces, refer to PPE indicated on door sign, to reduce the likelihood of cross contamination and for spill clean-up as determined by risk assessment.
Closed front Gowns	May be required in certain laboratory spaces, refer to PPE indicated on door sign or lab SOP, to reduce the likelihood of cross contamination.
Disposable lab coats / Gowns	May be required in certain animal laboratory spaces, refer to PPE indicated on door sign. May also be used as an alternative to cloth lab coats and gowns or when laundry service is unavailable.

EHS can assist in the correct selection of PPE. Laboratory dress code includes wearing long pants or equivalent leg covering and appropriate closed toe shoes.

Proper Use and Care of PPE

- Change gloves when the gloves are contaminated, the integrity has been compromised, or when otherwise necessary
- Do not wash or reuse disposable gloves.
- Remove PPE and wash hands when work has been completed with infectious materials and before leaving the lab. Dispose of PPE with other contaminated laboratory waste appropriately.
- Reusable PPE **must** be decontaminated prior to reuse.

Posting - Labeling and Storage

The necessity for establishing policies and procedures for proper identification of hazardous biological agents within U-M laboratories is to alert support and emergency personnel who may enter the area to take precautionary measures and to restrict traffic to potentially hazardous areas.

Signs

All areas and laboratories that contain biohazardous agents **must** be posted with a lab door sign. Contact EHS to obtain a lab-specific door sign using the appropriate form:

- [Laboratory Door Sign Request Form – Single Lab Director](#)
- [Laboratory Door Sign Request Form – Multiple Lab Directors](#)

A biohazard label, incorporating the universal biohazard symbol, should be placed on the face of these signs. These signs shall:

- Indicate the biosafety level of the laboratory.
- List the name and telephone number for the PI to facilitate contact in case of emergency.
- List the required procedures for entering and exiting the laboratory.

Labels and Tags

The universal "Biohazard" warning labels **must** be used to identify the following items:

- Containers of infectious materials; including waste and storage
- Refrigerators
- Incubators and/or freezers where biohazards are stored
- Equipment which may be contaminated through normal use of biohazards
- Laboratory animals (cages) which are potentially infectious

Storage of Biohazardous Materials

All infectious materials to be stored **must** be clearly labeled with the universal biohazard symbol. Additional information including contact name and emergency numbers **must** be visible on the refrigerator or freezer in case of emergency, i.e., freezer breakdown.

Materials for long-term storage **must** be annually inspected and each container **must** be checked for cracks and other damages and properly disposed or replaced. Expired and other unwanted material **must** be decontaminated properly.

In the event of a freezer melt-down, all materials that are unable to be salvaged **must** be properly treated by autoclaving or chemical disinfection prior to final disposal.

Audit Management

Maintaining accurate records and documentation is a critical part of any Biosafety Program. In order to prove that specific requirements of the Biosafety Program have been accomplished, appropriate documentation **must** be filed. Documentation is required for the following items:

RECORDS OF...	USE THE FOLLOWING DOCUMENTS...
Biosafety training	Documentation should be available to prove that laboratory personnel have been trained in the proper use of the specific biohazards with which they work. Documentation should be provided for new personnel and to document retraining (See Appendix 4)
Accident Investigation and Injury Illness Recordkeeping	http://www.umich.edu/~connect/pdf/iirf.pdf
Inspection/Audit Reports	Self-inspection and follow-up reports should be maintained for at least one year. Biological Safety Level 2 Inspection

SECTION 7: EQUIPMENT

Pipettes

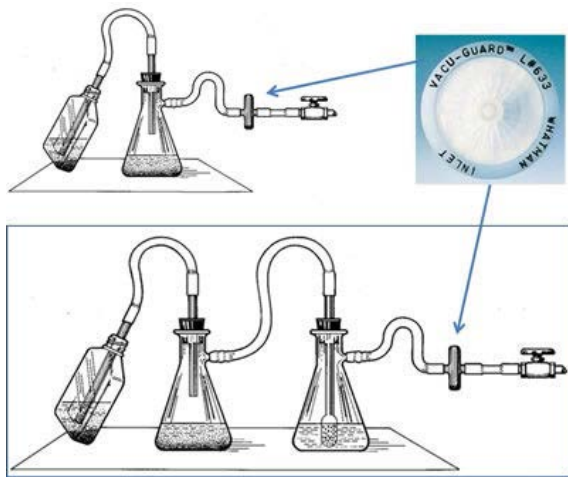
Filtered pipettes or tips should be used when pipetting biohazards, and whenever possible, glass pipettes should be replaced with disposable options.

Exposure to aerosols may occur when liquid from a pipette is dropped onto the work surface, when pipetting liquid cultures, or when the last drop of an inoculum is blown out. The safe pipetting techniques, which follow, are required to minimize the potential for exposure to hazardous materials.

- Mouth pipetting is prohibited.
- Contaminated pipettes should be collected for proper disposal.
- When resuspending liquid cultures, use a swirling action to create a homogeneous suspension with a minimum of aerosolization.

In-Line HEPA filter - Protection of Vacuum Line

Vacuum lines should be protected by a high efficiency particulate air (HEPA) vacuum filter (ex. VacuShield product). Use a liquid vacuum flask(s) to collect waste. These filters can isolate and confine infectious materials, preventing fluid and aerosol contamination of vacuum pumps or aspiration suction systems. Filters are available through laboratory supply catalogs. A second vacuum collection flask may be used as a backup (see below). If this set up is placed outside of the biosafety cabinet, it should be contained in a tray or pan to prevent accidental spills.



Syringes and Needles

The use of needles and syringes should be restricted to procedures for which there is no alternative. Needles and syringes should never be used as a substitute for pipettes. When needles **must** be used, the following procedures are recommended:

- Use safer needle devices such as: retractable device, needle locking mechanisms, etc.
- Bending, recapping, or removal of needles from syringes is prohibited. If it is essential that a needle be recapped the use of a mechanical device or the one handed scoop method **must** be used. This will require a lab-specific [Needle Recapping & Handling SOP](#). Labs **must** customize this SOP to reflect the reasons for recapping needles and how laboratory personnel will perform this procedure.
- Sharps container **must** be located close to the use area.
- Use a hard walled container of disinfectant for reusable needles such as Hamilton syringes. Do not place reusable needles in pans containing pipettes or other glassware in order to eliminate sorting later.
- Use approved puncture resistant sharps container for disposal.

Biological Safety Cabinet

The biological safety cabinet (BSC) is the primary engineering control used to provide containment of infectious aerosols generated by many microbiological procedures.

To assure sterility inside the cabinet and establish proper air flow for containment, the blower should be turned on at least 10 minutes before infectious materials are to be put in the BSC. Check to ensure that the airflow gage falls within the posted safe range on certification sticker before working in the hood. Airflow alarms are present on all cabinets.

NOTE: If airflow is incorrect, discontinue work and contact EHS at 647-1143. Make sure that all biohazard materials are properly secured and notify the PI or Laboratory Manager.

Before using the BSC, wipe down the interior with appropriate disinfectant to avoid accidental exposure to potentially infectious agents and prevent contamination of cultures. If 10% bleach is used, follow with 70% ethanol to prevent pitting of the stainless steel.

All materials should be placed as far back in the cabinet as practical, toward the rear edge of the work surface and away from the front and back grilles of the cabinet. NEVER place anything over the front

grille (paper, lab **NOTE**books, electronic devices, etc.) of a cabinet. Any disruption of the airflow in the cabinet decreases its effectiveness.

The biological safety cabinet **must** be disinfected with the appropriate disinfectant after each use.

Reference link below for more information on biosafety cabinet use:

[Working Safely in a Biological Safety Cabinet](#)

Centrifuges

Centrifugation of biohazardous material shall be done using centrifuge buckets with safety features such as biocontainment lids aka safety cups/rotors with lids. Each person operating a centrifuge should be trained on the proper operating procedures. Improperly used or maintained centrifuges can lead to equipment failure that present significant hazards to users. The high-speed spins generated by centrifuges can create large amounts of aerosol if a spill, leak, or tube breakage occurs. In the case of equipment failure, pieces of equipment can become projectiles causing injury to laboratory personnel and damage to the lab.

The following procedures for centrifugation are recommended:

1. Fill and decant all centrifuge tubes and bottles within the BSC. Avoid filling tubes to the rim. The maximum for centrifuge tubes is $\frac{3}{4}$ full. Wipe outside of tubes with disinfectant before placing in safety cups or rotors.
2. Use screw top caps on centrifuge tubes.
3. After samples to be centrifuged are prepared, load tubes into buckets inside the biological safety cabinet and seal carefully before moving to centrifuge.
4. After centrifugation, let the samples set for a minute to enable aerosolized particles to settle.
5. Open buckets in a biological safety cabinet to prevent exposure from aerosolized particles.

Blenders, Ultrasonic Disrupters, Grinders and Lyophilizers

The use of any of these devices results in considerable aerosol production. Blending, cell-disrupting, and grinding equipment **must** be used in a BSC when working with biohazardous materials. If the equipment does not fit inside of the BSC, contact EHS-Biosafety@umich.edu to complete a risk assessment and create a hazard-mitigation plan.

Microscopes

Tighten caps on flasks of infectious culture before transporting to the microscope. Infectious cultures in plates or other containers without tight fitting lids **must** be carried to the microscope in a sealed container. Disinfect the viewing platform of the microscope after each use.

Microtomes

Microtome blades are extremely sharp and **must** be handled with great care and stored safely when not in use. When changing the knife, stainless steel mesh gloves should be worn.

If the knife projects beyond the sectioning area, a suitable guard **must** be fitted. Always carry the knife, in its case, to the microtome. Never leave the knife on a microtome.

After use, always return the knife to its case or dispose of immediately. Slide the "back" on to the knife before removing it. Disinfect the microtome by wiping with bleach or sodium hydroxide solution.

Cryostats

- Frozen sections of unfixed human tissue or animal tissue infected with a biohazardous material pose a risk of infection. Freezing tissue does not inactivate all biohazardous materials.
- Freezing propellants under pressure should not be used for frozen sections as they may cause spattering of droplets of infectious material.
- Gloves should be worn during preparation of frozen sections.
- Consider the contents of the cryostat, including trimmings and sections of tissue, to be contaminated and decontaminate it frequently with 70% ethanol.
- Remove trimmings and sections with forceps during decontamination.
- Defrost and decontaminate the cryostat with a tuberculocidal disinfectant as needed.
- Handle knives with extreme care. Stainless steel mesh gloves should be worn when changing knife blades.

Bunsen Burners (on bench top)

Bunsen burners used on the bench for sterilization of inoculating loops or needles in an open flame **must** be handled with use and care to ensure safe handling. Stabilize the alcohol container so that it cannot tip over.

- Reduce the amount of flammable chemicals, equipment, and supplies in the work area. Use only enough alcohol for the experiment or technique.
- Have a “snuffing” lid available in case the alcohol in the container catches fire. Water is not a good choice for putting out fires.
- If you smell gas, turn off the exterior gas valve and wait until the gas has fully dissipated before lighting any flames.

In place of Bunsen burners, consider using a shielded electric incinerator or hot bead sterilizer. Disposable plastic loops and needles are also good alternatives to reduce generation of aerosols.

Open Flames in Biosafety Cabinets

Open flames inside a biosafety cabinet are **NOT** permitted.

Open flames create airflow turbulence that may compromise sterility and worker protection and heat buildup may damage the HEPA filters. Open flames are extremely dangerous around flammable materials, such as ethanol, which is often found in BSCs. Follow these tips for avoiding fires in your BSC:

- Use disposable pre-sterilized loops and spreaders.
- Replace Bunsen burners with alternative technology such as electric loop sterilizers, shielded electric incinerator, or hot bead sterilizer.

Equipment Maintenance

- Autoclaves, centrifuges, biological safety cabinets, and fume hoods should undergo regular preventative maintenance by qualified personnel.
- The airflow **must** be regularly checked on the biological safety cabinets and the filters changed by qualified personnel. If cabinets are not functioning correctly, contact EHS (734) 647-1143 to perform maintenance operations.
- Preventative maintenance records should be kept on all equipment.

Other Hazards

Dry Ice

Dry ice is the solid form of carbon dioxide, non-combustible, available in flakes, pellets or block form. It will sublime (vaporizes directly to the gas state) at a temperature of -78.5C (-109.3 F) or higher. Dry ice is commonly purchased from a commercial manufacturer. Dry ice is commonly used to cool reactions or to ship biological specimens.

All U-M laboratory personnel **must** follow the safe storage, usage, and handling of dry ice. U-M laboratory personnel responsible for shipping packages containing dry ice **must** be properly trained according to Department of Transportation (DOT) and International Air Transport Association (IATA) Regulations. <http://ehs.umich.edu/research-clinical/biological/transporting-biological-materials/>

Storage

- Dry ice is to be stored in a well-ventilated location and placed in a Styrofoam chest, insulated cooler, or a special cooler designed for the storage of dry ice.
- Because of the thermal expansion of dry ice (one pound of dry ice produces about 250 liters of gaseous carbon dioxide), sufficient gaseous carbon dioxide can be released in a sealed container to cause an explosion. Dry ice is NEVER to be stored in any type of tightly sealed devices such as an ultra-low freezer or plastic/glass container.
- Dry ice will sublimate about five to ten pounds every 24 hours (blocks last longer) in a typical storage cooler. Plan on purchasing dry ice as close as possible to the time needed.
- Normal air is composed of 78% nitrogen, 21% oxygen, and only 0.04% carbon dioxide. Concentrations greater than 0.5% (5000 ppm) can become dangerous. Therefore, handle dry ice in well-ventilated locations.

Hazards/Precautions:

- Burns/frostbite: Dry ice can cause burns to the skin in a short period. Thermal gloves are to be used if it is necessary to handle dry ice.
- Suffocation: Carbon dioxide is a simple asphyxiant. Always store dry ice in a well-ventilated area to minimize the buildup of carbon dioxide. Personnel **must** use caution should dry ice be stored in a deep cooler. Personnel **must** never stick one's head into the chest to obtain the dry ice.
- Explosions: Placing dry ice into a tightly sealed container can permit sufficient gas build up to cause an explosion. Never place dry ice inside an ultra-low freezer or other enclosed space.
- Placement of dry ice in rooms with little or no ventilation can result in a build-up of the carbon dioxide in the area. Do not store dry ice in a confined area such as walk-in coolers, refrigerators, freezers, closets, or cars/vans.
- When using dry ice to ship materials, the shipper **must** abide to all applicable shipping regulations.

Disposal

- Dispose unneeded dry ice by letting the unused portion sublime (recommended for well-ventilated locations because it will occur over a period of several days and the ventilation will take care of the gas liberated).
- NEVER dispose of dry ice in a sink, toilet or other drain (such action can destroy the structure because of the temperature difference).
- NEVER dispose of dry ice in the trash or garbage.
- NEVER place unneeded dry ice in corridors (some corridors may not be well ventilated and the oxygen level can be reduced to low levels).

Ultraviolet Light

The Center for Disease Control (CDC) and the National Institutes of Health (NIH) agree that UV lamps are not recommended nor required in a BSC. Proper use and cleaning of BSC negates any need for the use of UV lamps, which require regular cleaning, maintenance, and monitoring for germicidal activity.

If the laboratory decides to use UV lamps, the following protective measures **must** be adhered to:

- UV lamps **must** be turned off when the room is occupied to protect eyes and skin from UV exposure, which can burn the cornea and cause skin cancer.
- Laboratory personnel **must** wear a protective face shield and cover exposed skin.

If using UV microscopes, determine whether laboratory personnel using the microscope and anyone else in the room **must** wear protective goggles or glasses.

Cleaning and Decontamination

- All areas of the laboratory **must** be kept clean and orderly.
- Dirt, dust, and clutter are safety hazards and are not consistent with acceptable biological research.
- Vacuum lines should be protected by a liquid disinfectant trap.
- Contaminated materials to be reused **must** be chemically disinfected or placed, untreated, in autoclave bin prior to autoclaving.
- Surfaces are to be decontaminated after each use.
- Appropriate disinfectants should be available specific to the agents in use. Ensure appropriate contact time for the disinfectant and biohazardous materials, follow manufacturer's recommendations.

NOTE: Recommended disinfectants include 10% bleach 70% ethanol, Lysol, Virex, and quaternary ammonia compounds.

SECTION 8: BIOHAZARDOUS WASTE

Biohazardous waste is divided into the following categories:

CATEGORY OF BIOHAZARDOUS WASTE	INCLUDES...
Solid Biohazardous Waste	Plastic plates, petri dishes, paper towels, gloves, pipette tips, plastic serological pipettes, etc. that have been used with or come in contact with biological material.
Liquid Biohazardous Waste	Culture broths, media, stock cultures, centrifuge supernatants, blood, and solutions containing recombinant and synthetic nucleic acid molecules or any liquids that contain or have come in contact with viable biological material.
Sharp Waste	Needles with or without attached syringes, razor blades, glass slides, glass vials, and anything that can puncture the skin that has been used with or come into contact with biological material

Preparing Biohazardous Waste for Collection

The U-M, EHS or an approved vendor collects and processes the biological waste generated at U-M. Lab directors (faculty/lab managers/supervisors) **must** follow the EHS protocols for proper disposal of biological waste to ensure regulatory compliance, maintain a safe work place, and protect the environment.

- [Biological Waste Disposal Information](#)
- [Laboratory Refuse Collection Poster](#)

Autoclave

- Steam Sterilization is defined as 121C for at least 15 minutes peak temperature. The standard autoclave “cycle” is at least 45 minutes.
- Personnel operating autoclave(s) **must** be properly trained in its use. This training is provided by the responsible person designated for each autoclave.
- [Autoclave Safety Training](#) is required and available through EHS course BLS013w.
- Biohazardous materials **must** not be placed in autoclaves overnight in anticipation of autoclaving the next day.
- Wrap packages to allow for steam penetration; aluminum foil does not allow steam penetration, and should **not** be used for wrapping.
- Do not overload the chamber.
- Avoid over packing of autoclave bags.
- Do not seal bags or close bottles and other containers tightly.
- Do not stack containers.
- Always place autoclave bags/containers in a secondary container when autoclaving.
- Label waste by writing lab name and date on autoclave tape.

The changes that are seen on autoclave indicator tapes following an autoclave cycle do not guarantee that the contents of containers are sterile: they indicate only that the tape on the outside of the packages has been exposed to a certain amount of heat or steam. Proper autoclave performance is essential for sterilization. The time required for effective sterilization depends on the size of the load, volumes of liquid and density of materials to be autoclaved. Assessing autoclave performance regularly (at least once per month) is critical, the use of a heat-resistant biological indicator (BI) such as *Bacillus stearothermophilus*, should be used to ensure that the cycle in use really achieves sterilization conditions.

Reference [Autoclave SOP](#) for additional information

SECTION 9: RESEARCH ANIMALS

Laboratory animal facilities, operational practices, and quality of animal care **must** meet applicable standards and regulations (e.g., [Guide for the Care and Use of Laboratory Animals and Laboratory Animal Welfare Regulations](#)). For information regarding working in field environments, please refer to the [EHS Field Research](#) page

There is potential for zoonotic disease transmission to individuals handling research animals, including non-human primates, wild caught animals and any tissues or biological samples derived from these particular animals. Refer to the [EHS Species Specific Risks](#) page for further information.

All research experiments involving animals **must** be conducted in accordance with the associated [Institutional Animal Care and Use Committee \(IACUC\)](#) approved protocol. Animal research that involves a hazard (biological, radiological, or chemical) **must** be reflected in the approved IACUC protocol. All work involving human derived substances, recombinant DNA and synthetic nucleic acid molecules, infectious agents, biological toxins, certain animal-derived substances, and transgenic animals **must** be registered and approved by the IBC prior to commencement of the experiment.

The IBC will make the final determination of laboratory and housing containment upon IBC review of the proposed work. The IBC has the authority to increase or lower containment levels based on risk assessment.

Animal containment facilities are designed to protect personnel from exposure to potentially infectious materials. The containment facilities follow ABSL2 containment requirements including, maintaining rooms under negative pressure relative to all entrances and exhausting room air to the outside.

All lab staff administering biological materials to animals or staff who will be handling animals following administration **must** complete the Unit for Laboratory Animal Management (ULAM) [Hazard Containment Lecture and Workshop](#).

When animals are administered biological materials, the animal handler **must** wear appropriate PPE as indicated in the IBC and IACUC protocol approvals. All disposable PPE is single use and **must** be disposed of in the designated waste container upon exiting the space. Protective eyewear including safety glasses or goggles **must** be decontaminated and remain in the room.

Restraint devices and practices should be used to reduce the risk of exposure during animal manipulation. Animals **must** be manipulated within BSCs whenever procedures with a potential for creating infectious aerosols or splashes are conducted. These procedures may include necropsy of infected animals, harvesting of infected tissues or fluid from animals, intranasal inoculation of animals, and manipulation of high concentrations or large volumes of biohazardous materials.

Equipment and surfaces **must** be decontaminated after use and floors **must** be regularly cleaned. All equipment and wastes **must** be decontaminated by autoclaving or by other appropriate means before leaving the facility or **must** be appropriately contained until this can occur.

The standard operating procedure for animals administered biological materials requiring ABSL2 housing are found on the [Animals Administered a Hazardous Substance Requiring Containment](#) web page.

SECTION 10: MEDICAL SURVEILLANCE

An appropriate medical surveillance program **must** be in place for all work that requires BSL2 containment, as determined by risk assessment. Laboratory personnel **must** be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory. Personnel using respirators **must** be enrolled in the EHS [Respiratory Protection Program](#).

The PI or supervisory personnel should ensure that medical staff are informed of potential occupational hazards associated with all research. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women of childbearing age should be provided information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to U-M OHS provider for appropriate counseling and guidance. Refer to the [Medical Surveillance](#) or [Animal Handler Medical Surveillance Program](#) web page for more information.

SECTION 11: EMERGENCY MANAGEMENT

Spill Response Procedures for Biohazardous Material

Spills and accidents should be immediately reported to the PI and EHS (734) 647-1143. EHS may be contacted for assistance with biohazardous material spills.

Spill in the Laboratory

1. To prevent lab staff from potential aerosol exposure, leave the room immediately, lock the door, post a warning sign and inform your supervisor. Wait at least 30 minutes before reentering the lab to allow dissipation of aerosol created by the spill. During this time, review clean-up procedures, assemble decontamination materials and PPE.
2. Don PPE; lab coat, gloves, and safety glasses.
3. Remove sharp objects using a mechanical means and place into a sharps container.
4. Carefully cover spilled material with a paper towel. After the paper towel is in place, wet with an appropriate disinfectant.
5. Allow 15-20 minutes of contact time.
6. Transfer all contaminated materials (paper towels, gloves, labware, etc.) into biohazard waste containers for disposal.
7. Wipe surrounding surfaces with disinfectant to cover all splash areas.
8. Place all remaining contaminated materials, including protective clothing, into an autoclave bag or biohazard waste container.
9. Wash hands with soap and water.
10. If a personnel exposure occurred, see information below and complete an Illness or Injury Report form.

Spill in the Biosafety Cabinet

NOTE: Leave the cabinet turned on.

1. Don double gloves, a lab coat, and eye protection if not already wearing them.
2. Carefully cover spilled material with a paper towel. After the paper towel is in place, wet with an appropriate disinfectant. Let stand 15-20 minutes, wipe up and wash surface with appropriate disinfectant.
3. Spray or wipe cabinet walls, other work surfaces, and equipment with the appropriate disinfectant
4. If necessary, flood the work surface, drain pan, and catch basin below the work surface with disinfectant. Allow at least 15-20 minutes of contact time.
5. Soak up the disinfectant and drain the catch basin into a container. Lift the front exhaust grille and tray and wipe all surfaces. Ensure that no foreign materials are blown into the area below the grille.
6. If a 10% bleach solution is used on metal surfaces, rinse with water or 70% ethanol after decontamination is complete.
7. If the spill overflows into the interior of the cabinet, more extensive decontamination of the cabinet may be necessary. Contact EHS (734) 763-6973 for decontamination of the cabinet.

Personnel Exposure

1. If personnel are contaminated, remove potentially contaminated garments at the BSC and decontaminate garments by saturation with 70% ethanol or place in autoclave bag for autoclaving.
2. Wash hands and other potentially exposed skin surfaces thoroughly with soap and water.
3. Don fresh PPE, return to worksite, and spray walls, liners, and equipment with an appropriate disinfectant. If clothing or shoes are contaminated, carefully remove and place in an autoclave bag or another container. Contaminated items can be decontaminated by treatment with appropriate disinfectant such as 10% Bleach or Lysol. Contaminated items may also be autoclaved or collected for disposal.
4. If there is an agent-specific protocol for exposures, follow that (e.g., HIV, Herpes B).
5. In the case of skin contact or injury with a contaminated instrument:
 - a. Thoroughly wash area with soap and water. Do not squeeze the wound to induce bleeding.
 - b. Avoid use of abrasive chemical soaps or disinfectant washes as they can cause skin abrasions and a possible additional route of entry for the agent.
 - c. For mucous membranes (e.g., eyes, mouth), flush for a minimum of 15 minutes.
4. Notify U-M- OHS for evaluation of exposure following:
 - a. Contact with mucous membranes
 - b. Contact with non-intact skin
 - c. Percutaneous exposure
 - d. Any type of exposure that involves concentrated virus
5. Following this, the following forms **must** be completed:
 - Accident-Illness Report Form (<http://www.workconnections.umich.edu/employees/work-related-illness-injury/step-one/>) submit to Work~Connections (within 24 hours).
 - Report incident to EHS via Incident and Near Miss Form <https://ehsa.oseh.umich.edu/EHSA/public/injuryillnesssubmit/injuryillnessinitialedit>. If you have questions, please call (734) 647-1143.

Treatment Facilities

- U-M OHS -- Campus Employees
Mon-Fri 7:30 am - 4:30 pm
After hours - go to UM Hospital Emergency Dept. – Urgent Care Clinic
C380 Med Inn building
1500 East Medical Center Drive, Ann Arbor (734) 764-8021
- University Health Services -- University students (non-life threatening conditions)
Mon-Fri 8 am – 4:30 pm, Sat 9 am – 12 pm
Contact for current hours as they may vary
207 Fletcher Street, Ann Arbor (734) 764-8320
- UMHS Emergency Department -- after clinic hours or on weekends
1500 East Medical Center Drive, Ann Arbor, (734) 936-6666

Exposures to Recombinant DNA:

1. Any situation involving recombinant DNA that poses a threat to an individual's health, safety, or welfare should be handled with the appropriate care including emergency response (911) if necessary.
2. Wash the area thoroughly with soap and water.

3. Cover the wound with a sterile dressing.
4. Following this, reporting **must** occur to:
 - PI or Director of Lab (immediately)
 - BSO (734) 647-3133 (immediately)
 - Complete an Accident-Illness Report Form (<http://www.workconnections.umich.edu/employees/work-related-illness-injury/step-one/>) submit to Work~Connections (within 24 hours)
 - IBC (may be reported through the Biosafety Officer)
 - NIH/OBA (report will be coordinated by the IBC)

Reporting and Recordkeeping

Accidents and spills occurring outside the biological safety cabinets will be reported and accident report forms will be filed under Appendix 7.

SECTION 12: TRANSPORTING BIOLOGICAL MATERIALS

The movement of biohazardous materials, chemicals, or research animals can fall under various federal and state regulations. U-M has put into place restrictions on the movement of these materials on university modes of public transportation such as the U-M buses, where individuals unfamiliar with the materials may be potentially exposed or have the perception of exposure.

For more information, refer to the [Transporting Biological Materials Webpage](#).

SECTION 13: SELECT AGENTS

Researchers who wish to obtain or transfer Select Agent pathogens or regulated amounts of Select Agent toxins, specifically listed by CDC and USDA, **must** contact EHS Biosafety at EHS-Biosafety@umich.edu for additional information and guidance.

Certain select agent materials that meet regulatory criteria are exempt from registration with the CDC/APHIS. Laboratories using quantities of toxins below federally established thresholds are required register with the IBC and complete the [EHS Toxin Declaration Form](#).

SECTION 14: REQUIRED EHS BINDER DOCUMENTS

1. [Roster of Approved Laboratory Personnel and Training Records](#)
2. Agent list from ([Research Agents](#) form or PI's [IBC summary](#))
3. [Biosafety Proficiency](#) form
4. [Pathogen Safety Data Sheets](#) for Infectious Agents Located in Laboratory (if applicable)
5. Standard Operating Procedure (Download template)
6. [Autoclave Use Log](#) (if applicable)
7. [Hepatitis B Vaccination Form \(PDF\)](#) (if applicable)

SECTION 15: GLOSSARY OF TERMS

TERM	DEFINITION
Biohazardous Material	Any material known to harbor organisms or agents capable of infecting or infesting human or animal hosts or causing environmental harm if released.
Biosafety	<p>Biosafety is a specialized practice for proper handling and working with biohazardous organisms or biological material, which may harbor biohazardous organisms. Biosafety fits neatly into the traditional programmed approach to safety:</p> <ul style="list-style-type: none"> • Administrative controls to standardize methods to reduce exposure risk • Mechanical engineering controls for containment of hazardous materials • Medical surveillance and PPE for potentially exposed laboratory personnel • Workplace monitoring to determine exposure levels
Biosafety Levels (BSL)	<p>Describe the combination of safety practices, safety equipment and facility design used to contain the hazards associated with specific risk groups of microorganisms and is based on risk assessment.</p> <p>Biosafety levels are different from risk groups; however, risk group information is critical in determining the correct biosafety containment level.</p>
Blood borne Pathogen	An agent known to be transmissible through contact with human blood, such as the human immunodeficiency virus (HIV) or the hepatitis B virus (HBV).
Containment	<p>Primary Containment – The protection of personnel and the immediate laboratory environment from exposure to biohazardous material is provided by both good microbiological technique and use of appropriate safety equipment.</p> <p>Secondary Containment – The protection of the environment external to the laboratory from exposure to infectious materials, is provided by a combination of facility design and operational practices</p>
EHS	University of Michigan, Department of Environment, Health & Safety
Infectious Substance	A viable micro-organism, or its toxin, which causes or may cause disease in humans or animals, and includes those agents listed in 42 CFR 72.3 or any other agent that causes or may cause severe, disabling, or fatal disease.
IBC	Institutional Biosafety Committee; The IBC is responsible for assessing the biosafety containment level for research involving recombinant DNA, synthetic nucleic (including human gene transfer studies), infectious agents, biological toxins, human-derived tissues, fluids and cells, certain animal-derived tissues, fluids and cells, federally-regulated

TERM	DEFINITION
	Select Agents, experiments with Dual Use Research of Concern potential, and research requiring BSL3 containment
Occupational Exposure	An exposure that may place personnel at risk of injury or infection is defined as percutaneous (e.g., a needle stick or cut with a sharp object), contact of mucous membranes, or contact of skin (especially when the exposed skin is chapped, abraded, or afflicted with dermatitis or the contact is prolonged or involving an extensive area) with blood, tissues, or other bodily fluids to which universal precautions apply. For the purpose of this manual, the occupational exposure must be to fluids or aerosols known to be infectious.
UMOR	University of Michigan Office of Research
Potentially Infectious Material	Any material, which may or is known to contain an etiologic agent of human or animal disease.
Principal Investigator/Lab Director	The University of Michigan faculty member responsible for the research underway in the laboratory. For the purposes of this protocol, the Principal Investigator is
Recombinant DNA Molecules	Molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell; or molecules that result from the replication of those described above.
IACUC	Institutional Animal Care and Use Committee; The IACUC ensures that the highest animal welfare standards are maintained along with the conduct of accurate, valid scientific research through the supervision, coordination, training, guidance, and review of every project proposed to include the use of vertebrate animals at the U M
Risk Groups	Are the classification of infectious microorganisms based on principle characteristics such as; route of transmission and severity of disease. See below
Standard Microbiological Practices (SMP)	Basic safe laboratory work protocols for working with biological materials based on containment level. The main objective of SMP is to provide safety controls needed to protect workers and the environment from contamination in the event that the agents are accidentally released from their primary container.