INSTITUTIONAL BIOSAFETY COMMITTEE

BIOSAFETY MANUAL

2014

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I. INTRODUCTION

This Biosafety Manual is applicable to all laboratory, research, teaching, service, and support activities that may involve exposure to biohazardous agents or materials or otherwise comes under the purview of the Institutional Biosafety Committee (IBC) at the University of Montana. Activities that are specifically addressed are those involving:

- Recombinant or synthetic nucleic acid molecules, including transgenic animals and experiments that may be exempt under the NIH Guidelines
- Infectious agents, including bacteria, viruses, fungi, protozoans, and prions, as well as viral vectors
- Toxins of biological origin
- Human or non-human primate tissue or fluids, cell lines transformed with mammalian viruses, and human cell lines

I.A. Regulatory Requirements and Guidelines

Guidelines developed by the National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC) form the basis for the biosafety practices included in this manual. Guidelines must be followed to ensure the health and safety of personnel as well as the continuation of grant funding from federal agencies.

The NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

- Mandates the establishment of an Institutional Biosafety Committee;
- Outlines roles and responsibilities of the Institutional Biosafety Committee; and
- Establishes practices, procedures, and conditions under which recombinant or synthetic nucleic acid molecules research must be conducted.

The companion guideline from CDC, Biosafety in Microbiological and Biomedical Laboratories (BMBL 5th edition) addresses the appropriate measures and facilities for work with all microbial agents, including:

- bacterial
- viral
- fungal
- parasitic
- prion
- viral vectors

The Occupational Exposure to Bloodborne Pathogens Standard from the federal and state Occupational Safety and Health Administration (OSHA) addresses appropriate measures for work with:

- human blood
- human body fluids
- human tissue
- human cell cultures

The Public Health Service regulation 42 CFR Part 72 Interstate Shipment of Etiologic Agents and parts of the Department of Transportation Hazardous Materials regulation 49 CFR, Parts 171 – 180 addresses the requirements for packaging and shipment of biomedical. Information

on shipping procedures that comply with these regulations is found in the UM Pathogen Shipping Manual.

The Institutional Biosafety Committee (IBC) provides oversight of UM's biological safety program.

1. Facilitates the registration of biological research by providing materials and information to Principal Investigators;
2. Reviews research involving recombinant or synthetic nucleic acid molecules and other potentially biohazardous agents, and approves those that comply with NIH and CDC guidelines and with University policy;
3. Adopts policies supporting the safe use of biological materials and the elimination or reduction of exposure to potentially biohazardous materials and agents; and
4. Addresses biosafety issues related to experimentally infected laboratory animals.

I.B. IBC Application Submission and Approval

Each Principal Investigator (PI) is responsible for the preparation of registration documents for all research involving potentially biohazardous materials, including the assignment of the required Biosafety Level to the proposed research. This includes research involving:

1. Recombinant or synthetic nucleic acid molecules, including transgenic animals housed at ABSL-2 and experiments that may be exempt under the NIH Guidelines
2. Infectious agents, including bacteria, viruses, fungi, protozoans, and prions as well as viral vectors
3. Toxins of biological origin
4. Human or non-human primate tissue or fluids, mammalian cell lines transformed with viruses, and human cell lines

The IBC will review all IBC applications and consider approval for those applications that are complete and that provide for safe handling of potentially biohazardous materials under the appropriate Biosafety Level (BSL).

I.C. Principal Investigator (PI) Responsibilities

1. The PI of each laboratory is responsible for submitting an IBC application for research involving biohazardous materials as described in Section I.A.
2. The PI of each laboratory is responsible for providing the appropriate training of personnel and for verifying each person's competence. Persons working with infectious agents or infected materials must be aware of potential hazards and must be trained and proficient in the practices and techniques required for handling such material safely.
3. The PI of each laboratory is responsible for providing appropriate safety equipment including personal protection equipment (PPE) such as gloves, lab coats, safety glasses or goggles, face shields and respirators and safety equipment such as biosafety cabinets (BSCs).

I.D. Laboratory Biosafety Levels

CDC and NIH have established four levels of biosafety based on the degree of hazard associated with a microbial agent. These four biosafety levels (BSL), BSL-1 to 4, require successively more stringent practices and facilities as work moves from the least restrictive,

BSL-1, to work at the highest hazard level of BSL-4. The requirements at each laboratory biosafety level can be found in the CDC-NIH publication "Biosafety in Microbiological and Biomedical Laboratories", 5th Edition. Refer to Section II of this manual for a general outline of good practices at BSL-1, BSL-2, and BSL-3.

1. CDC Biosafety Levels
   - BSL-1: Agents not associated with disease in healthy adult humans.
   - BSL-2: Agents associated with human disease that are rarely serious and for which preventive or therapeutic interventions are often available.
   - BSL-3: Agents associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk).
   - BSL-4: Agents associated with life-threatening disease that is frequently fatal, for which there are no vaccines or treatments, or a related agent with unknown risk of transmission (no BSL-4 work is conducted at UM).
2. Animal Biosafety Levels

   Similar biosafety levels are provided for work with vertebrate animals infected with agents that may cause disease in humans. At UM only ABSL-1 and 2 facilities are available. These animal biosafety levels provide for practices, equipment, and facilities that are comparable to the laboratory biosafety levels. However, there are unique hazards associated with infected animals that must be understood by those personnel with animal contact and addressed in the animal facility. Animal activity can create aerosols, and bites and scratches can occur. A good summary of animal biosafety levels can be found in the CDC-NIH publication "Biosafety in Microbiological and Biomedical Laboratories", 5th Edition.

II. LABORATORY PROCEDURES AND EQUIPMENT

II.A. Guidelines for Good Laboratory Practices at BSL-1

1. General BSL-1 Information
   a. 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.
   b. Work is typically conducted on open bench tops using standard microbiological practices.
   c. Special containment equipment, such as a BSC, or facility design is not required.
   d. Lab personnel must have specific training in the procedures conducted in the laboratory.
   e. Lab personnel must be supervised by a scientist with training in microbiology or a related science.
2. IBC approval
   a. Before beginning work at BSL-1, obtain approval through the Institutional Biosafety Committee (IBC) for any work with recombinant or synthetic nucleic acid molecules or pathogenic agents.

b. Standard Operating Procedures for such work should be reviewed and signed by all lab personnel.

3. Standard Microbiological Practices
    a. Wash hands after working with potentially hazardous materials and before leaving the lab.
    b. Do not eat, drink, smoke, chew gum, handle contact lenses, or apply cosmetics in the laboratory. Persons wearing contact lenses in the laboratory should also wear goggles or a face shield. Do not bring any food, medications, or cosmetics into the laboratory for storage or later use. Food must be stored outside the work area in cabinets or refrigerators designated specifically for that purpose
    c. Mouth pipetting is prohibited; use mechanical pipetting devices.
    d. Establish and follow policies for safe handling of sharps.
    e. Perform all procedures carefully to minimize the creation of splashes or aerosols.
    f. Decontaminate equipment and work surfaces at completion of work, at the end of the day, and following spills of viable materials with appropriate disinfectant.
    g. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method.
    h. Post a sign incorporating the universal biohazard symbol at the entrance to the laboratory when infectious agents are present.
    i. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precaution to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates.
4. Safety Equipment (Primary Barriers and Personal Protective Equipment)
    a. Use of lab coats, gowns, or other designated laboratory uniforms is recommended to prevent contamination or soiling of street clothing.
    b. Wear protective eyewear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials.
    c. Gloves must be worn to protect hands from exposure to hazardous materials.
5. Laboratory Facilities (Secondary Barriers)
    a. Labs should have doors for access control.
    b. Labs must have a sink for hand washing.
    c. Bench tops must be impervious to water and resistant to heat and chemicals.
    d. Chairs used for lab work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

II.B. Guidelines for Good Laboratory Practices at BSL-2

BSL-2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment.

1. General information specific for BSL-2
    a. Lab personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures
    b. Access to the laboratory is restricted when work is being conducted

c. All procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

2. IBC approval
    a. Before beginning work at BSL-2, obtain approval through the Institutional Biosafety Committee (IBC) for any work with recombinant or synthetic nucleic acid molecules, pathogenic agents, human blood or human cell lines.
    b. Standard Operating Procedures for such work should be reviewed and signed by all lab personnel.
3. Standard Microbiological Practices for BSL-2
    a. Access to the laboratory is limited or restricted by the PI when experiments are in progress.
    b. Wash hands after working with potentially hazardous materials and before leaving the laboratory.
    c. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the laboratory. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Store food outside the work area in cabinets or refrigerators designated for this purpose only.
    d. Mouth pipetting is prohibited; use mechanical pipetting devices.
    e. Policies for the safe handling of sharps are instituted.
        - Use a high degree of caution when handling any contaminated sharp item, such as needles and syringes, slides, pipettes, capillary tubes, and scalpels;
        - Restrict needles and syringes or other sharp instruments in the laboratory for use only when there is no alternative, such as for parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles.
        - Substitute plasticware for glass whenever possible.
        - Handle broken glassware with a brush and dustpan, tongs or forceps, not directly with hands.
        - Use only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) for injection or aspiration of infectious material. Syringes which re-sheathe the needle, needle-less systems and other safety devices should be used whenever possible and appropriate; and
        - Do not bend, shear, break, recap, or remove used needles from disposable syringes or otherwise manipulate such units by hand before disposal. Dispose of needles and syringes in the puncture resistant sharps container provided in the laboratory for this purpose. Call EHRM for pick up (do not discard into trash).
    f. Perform all procedures carefully to minimize the creation of aerosols.
    g. Decontaminate work surfaces at least once a day and after any spill of viable material.
    h. Decontaminate all cultures, stocks, and other regulated wastes before disposal by an approved decontamination method, such as autoclaving.

i. Ensure that when biohazardous agents are in use or stored in the laboratory, a biohazard sign is posted on the lab access door. This sign identifies the agent(s) in use, the biosafety level, any required immunizations, the PI's name and telephone number, and any PPE that must be worn in the laboratory

j. The PI must ensure that all laboratory personnel receive appropriate training on hazards associated with the agents involved, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive training before starting work with the agents, as well as annual updates and additional training as necessary for procedural or policy changes. The laboratory director is responsible for ensuring that all personnel demonstrate proficiency in standard microbiological practices and techniques before working with organisms at BSL- 2.

4. Special Practices
    a. All persons entering the lab must be advised of the potential hazards.
    b. Lab personnel must be provided medical surveillance, as appropriate, and offered available immunizations for agents handled.
    c. A lab-specific biosafety manual must be prepared and adopted as policy.
    d. Lab personnel must demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.
    e. Potentially infectious materials must be placed in a durable, leak proof container for transport within a facility.
    f. Lab equipment should be routinely decontaminated.
        - Decontaminate equipment and work surfaces at completion of work, at the end of the day, and following spills of viable materials.
        - If a spill occurs, cover the spill with paper towels and soak the towels with a 1 to 10 dilution of chlorine bleach (made fresh; see Appendix A) or other suitable disinfectant. Allow the material to soak for approximately 20 minutes before discarding materials in biohazard bag.
    g. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the lab biosafety manual. All incidents must be reported to the lab supervisor.
    h. All procedures involving the manipulation of infectious material that may generate an aerosol should be conducted within a BSC or other physical containment device.
5. Safety Equipment (Primary Barriers and Personal Protective Equipment)
    a. Use BSCs whenever:
        - Procedures with a potential for creating infectious aerosols or splashes are conducted.
        - High concentrations or large volumes of infectious agents are used.
    b. Wear lab coats, gowns, smocks, or other provided protective garments while in the lab. Remove lab coats and other protective clothing when exiting the lab and for disposal or laundering

c. Wear appropriate face protection (goggles, mask, face shield or other splatter guard) for anticipated splashes or sprays of biohazardous agents to the face when agents must be handled outside the BSC.
d. Gloves must be worn to protect hands from exposure to hazardous materials.
    - Gloves must not be worn outside the laboratory.
    - Do not wash or reuse disposable gloves
    - Wash hands after removing gloves
e. Wear appropriate face protection (goggles, mask, face shield or other splatter guard) for anticipated splashes or sprays of biohazardous agents to the face when agents must be handled outside the BSC.

6. Laboratory Facilities (Secondary Barriers)
    a. Lab must have a sink for hand washing.
    b. Lab furniture must be capable of supporting anticipated loads and uses.
        - Bench tops must be impervious to water and resistant to heat and chemicals.
        - Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
        - 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 and heavily traveled laboratory areas, and other possible airflow disruptions.
        - Vacuum lines should be protected with liquid disinfectant traps
        - An eye was station must be readily available.
    c. A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, etc.).
    d. Dispose of all biohazard wastes and associated wastes into biohazard bags and autoclave before disposal.
    e. Cover all containers of cultures, tissues, specimens of body fluids, or other potentially infectious waste to prevent leakage during collection, handling, processing, storage, transport, or shipping

II.C. Guidelines for Good Laboratory Practices at BSL-3

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents that may cause serious or potentially lethal disease, usually as a result of exposure by the inhalation route. The following standard and special safety practices, equipment and facilities apply to agents assigned to BSL-3.

1. General information specific for BSL-3
    a. Laboratory personnel must have specific training in handling pathogenic and potentially lethal agents and they are supervised by competent scientists who are experienced in working with these agents
    b. Access to the laboratory is restricted when work is being conducted
    c. All procedures involving the manipulation of infectious materials must be conducted within biological safety cabinets (BSCs) or other physical containment

devices, and by personnel wearing appropriate personal protective clothing and equipment.

d. The laboratory has special engineering and design features.

2. IBC approval
    a. Before beginning work at BSL-3, obtain approval through the Institutional Biosafety Committee (IBC) for any work with recombinant or synthetic nucleic acid molecules, pathogenic agents, human blood or human cell lines.
    b. Standard Operating Procedures for BSL-3 work should be reviewed and signed by all lab personnel.
    c. Currently, Select Agents are NOT approved for use at UM.
3. Standard Microbiological Practices for BSL-3
    a. Access to the laboratory is limited or restricted by the PI when experiments are in progress. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures, enter the laboratory.
    b. Wash hands after handling infectious materials, after removing gloves, and when leaving the laboratory.
    c. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the laboratory. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Store food outside the work area in cabinets or refrigerators designated for this purpose only.
    d. Use mechanical pipetting devices; do not pipette by mouth
    e. Institute policies for the safe handling of sharps.
    f. Perform all procedures to minimize the creation of aerosols.
    g. Decontaminate work surfaces at least once a day and after any spill or splash of viable material with appropriate disinfectant.
    h. Decontaminate all cultures, stocks, and other regulated wastes before disposal by an approved decontamination method, such as autoclaving. Nothing leaves the BSL-3 without first being decontaminated.
    i. Post a sign incorporating the universal biohazard symbol at the entrance to the laboratory. The hazard warning sign identifies the agent, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates any special requirements for entering the laboratory, such as the need for immunizations, respirators, or other personal protective measures
    j. The lab supervisor must ensure that lab 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.
        - Personnel should be provided with information regarding immune competence and conditions that may predispose them to infection.
4. Special Practices

a. All persons entering the lab must be advised of the potential hazards and meet specific entry/exit requirements.
b. Lab personnel must be offered appropriate immunizations for agents handled or potentially present in the lab.
c. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
d. The lab supervisor must ensure that lab personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents.
e. Potentially infectious materials must be placed in a durable, leak proof container during collection handling, processing, storage, or transport within a facility.
f. Lab equipment should be routinely decontaminated, as well as after spills, splashes.
g. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the lab biosafety manual. All such incidents must be reported to the lab supervisor. Medical evaluation and treatment should be provided and appropriate records maintained.
h. All procedures involving the manipulation of infectious materials must be conducted within a BSC or other physical containment devices.

5. Safety Equipment (Primary Barriers and Personal Protective Equipment)
    a. All manipulations of infectious materials are conducted in a Class II biosafety cabinet. Refer to "Biological Safety Cabinet" Section III.E-G of this manual for information on classification and use of biosafety cabinets.
    b. Wear protective laboratory clothing such as solid-front or wrap-around gowns, scrub suits, or coveralls when in the laboratory. Do not wear protective clothing outside the laboratory. Decontaminate reusable clothing before being laundered. Change clothing when it is overtly contaminated.
    c. Gloves must be worn to protect hands from exposure to hazardous materials.
        - Change gloves frequent accompanied by hand washing.
        - Do not wash or reuse disposable gloves.
        - Wear two pairs of gloves when appropriate.
        - Wash hands before leaving the laboratory.
6. Laboratory Facilities (Secondary Barriers)
    a. The laboratory is separated from areas that are open to unrestricted traffic flow within the building, and access to the laboratory is restricted. Passage through a series of two self-closing doors is the basic requirement for entry into the laboratory from access corridors. Doors are lockable.
    b. Each laboratory room must have a sink for hand washing. The sink is hands-free or automatically operated and is located near the room exit door.
    c. The interior surfaces of walls, floors, and ceilings of areas where BSL-3 agents are handled must be constructed for easy cleaning and decontamination. Seams, if present, must be sealed. Walls, ceilings, and floors should be smooth, impermeable to liquids and resistant to the chemicals and disinfectants normally used in the laboratory. Floors should be monolithic and slip-resistant.

d. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and those chemicals used to decontaminate the work surfaces and equipment.
e. Laboratory furniture must be capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.
f. All windows in the laboratory are closed and sealed.
g. A method for decontaminating all laboratory wastes is available in the facility and utilized, preferably within the laboratory (i.e., autoclave, chemical disinfection, incineration, or other approved decontamination method). Consideration should be given to means of decontaminating equipment. If waste is transported out of the laboratory, it should be properly sealed and not transported in public corridors.
h. Biological safety cabinets are required and are located away from doors, from room supply louvers, and from heavily traveled laboratory areas.
i. A ducted exhaust air ventilation system is provided. This system creates directional airflow that draws air into the laboratory from "clean" areas and toward "contaminated" areas. The exhaust air is not recirculated to any other area of the building. Filtration and other treatments of the exhaust air are not required, but may be considered based on site requirements, and specific agent manipulations and use conditions. The outside exhaust must be dispersed away from occupied areas and air intakes, or the exhaust must be HEPA-filtered. Laboratory personnel must verify that the direction of the airflow (into the laboratory) is proper. A visual monitoring device that indicates and confirms directional inward airflow is recommended at the laboratory entry. Consideration should be given to installing an HVAC control system to prevent sustained positive pressurization of the laboratory. Audible alarms should be considered to notify personnel of HVAC system failure.
j. HEPA-filtered exhaust air from a Class II biological safety cabinet can be recirculated into the laboratory if the cabinet is tested and certified at least annually. When exhaust air from Class II safety cabinets is to be discharged to the outside through the building exhaust air system, the cabinets must be connected in a manner that avoids any interference with the air balance of the cabinets or the building exhaust system (e.g., an air gap between the cabinet exhaust and the exhaust duct).
k. Continuous flow centrifuges or other equipment that may produce aerosols are contained in devices that exhaust air through HEPA filters before discharge into the laboratory. These HEPA systems are tested at least annually. Alternatively, the exhaust from such equipment may be vented to the outside if it is dispersed away from occupied areas and air intakes.
l. Vacuum lines are protected with liquid disinfectant traps and HEPA filters or their equivalent. Filters must be replaced as needed. An alternative is to use portable vacuum pumps (also properly protected with traps and filters).

m. An eyewash station is readily available inside the laboratory.

II.D. Biological Safety Cabinets (BSCs)

BSCs used at UM are classified as Class I or Class II cabinets. When properly maintained and operated, they effectively contain and capture microbial contaminants and infectious agents using HEPA (High Efficiency Particulate Air) filters.

- Biosafety cabinets should not be confused with clean benches that only protect the material being worked with and are not suitable for work with infectious or toxic material. (Although clean benches, like BSCs, have HEPA-filtered air, the air flows in clean benches over the experimental material toward the user rather than being drawn away.)
- BSCs should also not be confused with conventional fume hoods that do not filter microorganisms.

1. Types of BSCs

   BSCs are classified as Class I, Class II or Class III cabinets. When properly maintained and operated, they effectively contain and capture microbial contaminants and infectious agents using HEPA (High Efficiency Particulate Air) filters. Refer to the BMBL, 5th Edition for details.

   a. Class I BSC: provides personnel and environmental protection, but not product protection. It has a HEPA filter in the exhaust system.
   b. Class II BSC (types A1, A2, B1 and B2): provide personnel, environmental and product protection. Exhaust air is HEPA filtered and may be recirculated to the laboratory or discharged from the building via a canopy connection (Type A1 and A2). Exhaust air from Types B1 and B2 must be discharged to the outdoors via a hard connection.
      - All Class II BSCs are suitable for for work involving microorganisms assigned to biosafety levels 1, 2 and 3.

2. Guidelines for Working in a BSC
   a. Turn off the ultraviolet lamp if one is in use. Turn on the fluorescent lamp.
   b. Inspect the air intake grilles for obstructions and foreign material and remove if necessary.
   c. Adjust view screen to proper height.
   d. Turn the cabinet on for at least 10 minutes prior to use, if the cabinet is not left running.
   e. Prepare a written checklist of materials necessary for the particular activity.
   f. Put on a long-sleeved gown or lab coat with tight-fitting cuffs. Coat should be buttoned. Put on a pair (or two pairs) of high quality nitrile gloves.
   g. Disinfect work surface with a suitable disinfectant.
   h. Place items into the cabinet so that they can be worked with efficiently without unnecessary disruption of the airflow, working with materials from the clean to the dirty side.
   i. Adjust the working height of the stool so that the worker's face is above the front opening.

j. Delay manipulation of materials for approximately one minute after placing hands/arms inside the cabinet.
k. Minimize the frequency of moving hands in and out of the cabinet.
l. Do not disturb the airflow by covering any portion of the grillwork with materials.
m. Work at a moderate pace to prevent the airflow disruption that occurs with rapid movements.
n. Wipe the bottom and side of the hood surfaces with disinfectant when work is completed

3. Certification of the BSC

Certification is a series of performance tests on the BSC to confirm that it will provide the user and experimental material the protection for which it is designed. The airflow, filters, and cabinet integrity are checked to ensure that the cabinet meets minimum performance standards. Certification is provided by an outside vendor such as ***Asepsis Air Control, Inc.*** [telephone: (866-782-5655)]. BSCs intended for user protection and/or BSL-2 work must be certified:

a. After they are received and installed (before use with infectious materials)
b. After filter changes
c. After being moved (even a few feet)
d. Annually

II.E. Decontamination

1. Definitions
   a. Decontamination is a process or treatment that renders an instrument or environmental surface safe to handle. A decontamination procedure can be as simple as cleanup with detergent and water or as thorough as sterilization. Sterilization, disinfection, and antisepsis are all forms of decontamination.
   b. Sterilization is the use of physical or chemical processes to destroy all viable forms of microbial life, including bacterial spores.
   c. Disinfection is the elimination of essentially all pathogenic non-spore forming microorganisms but not necessarily all microbial forms from work surfaces and equipment. Effectiveness is influenced by a number of factors, including types and numbers organisms; amount of organic matter; the object being disinfected; the disinfectant being used; exposure time, temperature and concentration.
   d. Antisepsis is the application of a liquid antimicrobial to skin or other living tissue to inhibit or destroy microorganisms. Examples include hand washing with germicidal solutions or swabbing skin with alcohol before an injection.
2. When to Decontaminate

In most UM laboratories, decontamination is accomplished by steam heat sterilization in an autoclave. All material and equipment contaminated with or containing potentially biohazardous agents should be decontaminated:

a. Upon completion of procedures involving the use of biohazardous agents or toxins;
b. In the event of spills of such materials;
c. At least daily when in use;

d. Before being washed, stored, or discarded.

3. Autoclave Use

   Autoclaving (saturated steam under pressure of approximately 15 pounds per square inch (psi) to achieve a chamber temperature of at least 121°C for a designated time is the preferred and most convenient method to rapidly destroy all forms of microbial life. However, to do this, the autoclave process must reach proper temperature, pressure, and time, and also prevent the entrapment of air in the bag or container of treated material.

   a. Material to be sterilized must come into contact with live steam.
   b. Bags or containers should be left open during autoclaving or water (~200 ml) should be added to sealed bags to generate steam.
   c. Heat indicator tape should be used inside the bag or container with each autoclave load to indicate that sterilization has been completed.
   d. Autoclave sterility monitoring should be conducted on a regular basis using biological indicators (such as *Bacillus stearothermophilus* spore strips) placed among treated materials and at locations throughout the autoclave. The spores, which are more resistant to heat than most microbes, provide validation of general microbial destruction when they are effectively inactivated by autoclave operation (typically 121°C for 30 minutes)

4. Chemical Disinfectant Use

   The most practical use of chemical disinfectants is for surface decontamination and, when used in sufficient concentration, as a decontaminant for liquid wastes prior to final disposal. General recommendations are:

   a. Liquid Decontamination
      - Add liquid chlorine bleach (see Appendix A) to provide a final 1:10 dilution (made fresh daily)
      - Let stand on surface for at least 20 minutes; and
      - Discard the solution appropriately
   b. Surface Decontamination
      - Wipe with 1:10 dilution of bleach (made fresh daily; see Appendix A); or
      - Wipe with iodophor disinfectant (per label concentration), or
      - Wipe with 70% alcohol

II.F. Exposure to Biohazard Agent

In the event of an exposure to a biohazard agent or material, the following guidelines should be used:

1. Intact Skin
   a. Remove contaminated clothing. Clothing should not be pulled over the face as contact with eyes, nose, and mouth may occur. Shirts should be cut off.
   b. Vigorously wash contaminated skin for 1 minute with soap and water.
   c. Call 911 or seek medical attention at Curry Health Center, if necessary.
   d. Inform the laboratory's PI, the Biosafety Officer and EHRM

2. Broken, Cut or Damaged Skin or Puncture Wound
    a. Remove contaminated clothing. Clothing should not be pulled over the face as contact with eyes, nose, and mouth may occur. Shirts should be cut off.
    b. Vigorously wash contaminated skin for 5 minutes with soap and water.
    c. Call 911 or seek medical attention at Curry Health Center, if necessary.
    d. Inform the laboratory's PI, the Biosafety Officer and EHRM.
3. Eyes
    a. Immediately flush eyes for at least 15 minutes with water, using an eyewash. (Hold eyelids away from your eyeball and rotate your eyes so that all surfaces may be washed thoroughly)
    b. Remove contaminated clothing. Clothing should not be pulled over the face as contact with eyes, nose, and mouth may occur. Shirts should be cut off.
    c. Call 911 or seek medical attention at Curry Health Center, if necessary.
    d. Inform the laboratory's PI, the Biosafety Officer and EHRM.
4. Ingestion or Inhalation
    a. Move to fresh air immediately.
    b. Call 911 or seek medical attention at Curry Health Center, if necessary.
    c. Do not induce vomiting unless advised to do so by a health care provider.
    d. Inform the laboratory's PI and EHRM
5. Biological Material Spills
   UM does not have a centralized biological spill response team. Therefore, each laboratory working with potentially hazardous biological material must be prepared and trained to handle biological spills. EHRM is available for assistance if necessary.

II.G. Spills and Preparing for Them

In the event of a spill of biological material, the individual(s) who caused the spill is responsible for the cleanup. If additional resources are needed, call EHRM at 243-4503 or refer to the emergency numbers posted in your lab.

1. General Information
    a. Minimize the consequences of any spill of biological material by performing all work on plastic-backed liner to absorb spills
    b. Have a simple spill kit on hand including:
        - Chlorine bleach or some other concentrated disinfectant
        - A package or roll of paper towels
        - Autoclavable bags
        - Disposable gloves
        - Forceps or broom and dust pan for picking up broken glass
2. Spills Inside a Biological Safety Cabinet
    a. Alert the other laboratory employees.
    b. Leave the cabinet turned ON.
    c. Wear gloves. Spray or wipe cabinet walls, work surfaces and equipment with disinfectant equivalent to 1:10 bleach solution (made fresh daily; see Appendix A). If necessary, flood the work surface, as well as drain-pans and catch basins below the work surface, with disinfectant for a contact time of at least 20 minutes.
    d. Report the spill to the laboratory's PI.

e. Soak up disinfectant and spill with paper towels. Drain catch basin into a container. Lift front exhaust grill and tray and wipe all surfaces. Ensure that no paper towels or solid debris have blown into the area beneath the grill.
f. Dispose of cleanup materials into the biohazard waste container.
g. Wash hands and any exposed surfaces thoroughly after the cleanup procedure
h. Autoclave all waste material.

3. Small Spill of Material Outside of a Biological Safety Cabinet (spill that can be covered by a few paper towels)
    a. Alert laboratory employees.
    b. Wear gloves, safety glasses and a lab coat. Cover the spill with paper towels and gently apply disinfectant, proceeding from the outer edge of the spill to its center. Leave in place for 20 minutes.
    c. Report the spill to the laboratory's PI.
    d. Pick up the towels and discard into a biohazard container. Pick up any pieces of broken glass with forceps and place in sharps container.
    e. Re-wipe the spill area with disinfectant and thoroughly wash hands after glove removal.
    f. Autoclave all waste material.
4. Large Spill of BSL-2 Material (>500 ml) Outside of a Biological Safety Cabinet
    a. Hold your breath and leave the room immediately.
    b. Warn others to stay out of the spill area to prevent spread of contamination.
    c. Post a sign stating: "DO NOT ENTER, BIOHAZARD SPILL, contact (name and phone #) for information".
    d. Remove any contaminated clothing, ensuring that clothing is not pulled over the face, and put into a biohazard bag for later autoclaving.
    e. Wash hands and exposed skin.
    f. Report the spill to the laboratory's PI.
    g. Put on protective clothing (lab coat, gloves and, if indicated, respirator, eye protection, shoe covers) and assemble cleanup materials.
    h. Wait 30 minutes before re-entering the contaminated area to allow for dissipation of aerosols.
    i. Cover the spill with paper towels and gently apply disinfectant, proceeding from the outer edge of the spill to its center. Leave in place for 20 minutes.
    j. Collect and autoclave all treated material and discard in a biohazard container. Pick up any broken glass with forceps and place them into a sharps container.
    k. Re-wipe the spill area with disinfectant and wash hands thoroughly at completion of cleanup.

II.H. Biological Agent Waste Handling

Wastes associated with biological materials must be specially disposed because they may have been contaminated with infectious agents. See Biological Waste Stream pages Section II.I in order to clarify how these various wastes are to be handled in laboratories using biological materials. These potentially infectious items include the following:

1. All sharps, e.g. glass implements, needles, syringes, blades, etc., coming from facilities using infectious materials.
2. Biologically-cultured stocks and plates.
3. Human blood or tissues.
4. Biological wastes derived from human sources such as blood, body fluids, tissues, tumors, human cell lines, etc. are hazardous biological wastes and should be placed in a red or orange biohazard bag. Place an “X” of autoclave tape over the biohazard symbol on red bag before autoclaving; after autoclaving place the autoclaved bag into regular trash (the biohazard symbol must be crossed out by the heat-sensitive autoclave tape or marked-out with a black marking pen before disposal into regular trash.
5. Bacteria, viruses, and other microorganisms that are known human pathogens should also be put into a red or orange bag, autoclaved under appropriate time and temperature. Place an “X” of autoclave tape over the biohazard symbol on red bag before autoclaving; after autoclaving place the autoclaved bag into regular trash (the biohazard symbol must be crossed out by the heat-sensitive autoclave tape or marked-out with a black marking pen before disposal into regular trash.
6. Sharps and sharp objects such as glass, syringes, and disposable pipettes that may be contaminated with biological waste (human or non-human) or pathogenic material should be placed in a rigid, leak-proof, puncture-resistant container. Place sharps into labeled sharps containers. When the sharps container is 2/3 full, call EHRM (243-4503) for pickup.
7. Animal bedding waste: Laboratory Animal Resources (LAR) staff will normally change all bedding for the researchers. Unless known to contain an infectious or toxic material, the bedding will be disposed of in the normal trash by the LAR staff. In the event the bedding represents an unusual risk, the LAR Director and the IBC will determine appropriate safeguards and collection and disposal practices in conjunction with the researcher.
8. Animal carcasses: Freezers are provided in each animal facility for storage of carcasses that have been bagged and sealed. The frozen carcasses are picked up on a regular schedule for appropriate disposal. Freezers are cleaned and defrosted as necessary by animal laboratory personnel to keep them in a sanitary condition.
9. Animal waste from ABSL-2: Animals housed in an ABSL-2 animal space are considered to be potentially infectious because as part of the research protocol they are infected with Biosafety Level 2 (BSL-2) animal and/or human pathogens. Animal bedding, carcasses, and tissue are placed in biohazard bags by the research or LAR staff. All animal bedding is autoclaved before being placed in the normal trash. Bagged animal carcasses and tissue are placed in the provided storage freezer and removed by LAR staff to medical waste boxes for pickup by EHRM as part of the medical waste stream.
10. All disposable wastes generated at Curry Health Center from patient rooms and as part of direct patient care are considered potentially infectious and are disposed of in the medical waste stream.
    a. Syringes, needles, and other sharps are placed in the provided sharps container which, when filled and sealed are placed in the provided medical waste boxes.
    b. Patient care waste generated at other sites on campus by medical response personnel (i.e., Public Safety) are placed in biohazard bags and brought to Curry

Health Center for medical waste disposal or handled by responding EHRM personnel.

c. A program is in place to ensure that needles and syringes generated as part of personal diabetes care will not be an exposure hazard to others. Collection containers are available from Curry Health Center that, when filled, are returned to Curry Health Center for proper disposal in the medical waste stream

II.I. Biological Hazard Waste Stream

All biohazard waste must be disposed of as outlined below. There are no exceptions.

1. Sharps: All sharps must be collected in durable containers for offsite disposal with our biohazard waste contractor. Special heavy wall red plastic biohazard sharps containers, coffee cans or other puncture proof, stiff walled containers may be used for sharps collection. Plastic milk bottles, cardboard boxes or other containers that are likely to allow a needle to penetrate the container walls during handling may NOT be used. Under no circumstances may sharps be disposed of in the normal trash. Sharps containers are picked up by EHRM upon request.
2. Liquid blood or other body fluids: Liquid blood or other body fluids may be disposed of down the drain. If you know these materials contain agents that are infectious via airborne transmission, you must disinfect them with a bleach solution prior to drain disposal.
3. Solid biohazard waste:
    a. Solid biohazard waste such as used bandages, gloves, counter pads, disposable labware, pipette tips, etc. may be treated in several different ways.
    b. Small quantities may be chemically disinfected and disposed of in the normal trash as long as no appreciable free liquid is present.
    c. Dry waste may be autoclaved and disposed of in the normal trash if the biohazard symbol is defaced on the bag prior to disposal. If biohazard bags are used that auto indicate that acceptable temperatures have been attained, then the bags and contents may be disposed of without manually defacing the biohazard symbol.

II.J. Incident Reporting

Supervisors must submit accident reports to the Biosafety Officer and to Environmental Health and Risk Management for any accident or near-miss situations. All employees will be free from any reprisals for reporting accidents. Laboratory near-miss reports, corrective actions, and suggestions can be helpful to safety committees to improve biological safety.

III. TRANSFERS, PACKAGING, AND SHIPPING OF BIOLOGICAL MATERIALS (refer to UM Pathogen Shipping Manual)

IV. BIOSECURITY

The objective of biosecurity is to prevent loss, theft or misuse of microorganisms, biological materials, and research-related information. This is accomplished by limiting access to facilities, research materials and information. Biosecurity is based upon risk assessment and management methodology; personnel expertise and responsibility; control and accountability for research materials including microorganisms and culture stocks; access control elements; material transfer documentation; training; emergency planning; and program management

V. INSPECTIONS AND COMPLIANCE

The UM Biosafety Officer (BSO) will conduct regular inspections, at least biennially, of each laboratory to ensure compliance with the procedures and protocols of this manual. Inspection reports will document problems and be directed to the PI. The results of these inspections will be documented and shared with the IBC and kept on file with the BSO.

VI. RECORDKEEPING

At a minimum, the PI must maintain the following records and be prepared to present these at the annual laboratory inspection:

1. An accurate and current list of each biological agent stored in that room.
2. Completed training documentation forms including immunizations or declinations.
3. Safety, security, and emergency response plans.
4. Safety and security incident reports.
5. Approved IBC application.

TABLE 1 - DISINFECTANT ACTIVITY**

Disinfectants			Practical Requirements				Inactivates				
Type	Category	Use Dilution	Contact Time (min Lipovirus)	Contact Time (min) Broad Spectrum	Temperature (C^o)	Relative Humidity (%)	Vegetative Bacteria	Lipoviruses Viruses	Nonlipid	Mycobacteria	Bacterial Spores
Liquid	Quat. Ammon Cpds	0.1%-2.0%	10	NE			+	+			
	Phenolic Cpds	1.0%-5.0%	10	NE			+	+	B		
	Chlorine Cpds	500 ppm*	10	30			+	+	+	+	+
	Iodophor	25-1600 ppm*	10	30			+	+	+		
	Alcohol, Ethyl	70%-85%	10	30			+	+	B		
	Alcohol, Isopropyl	70%-85%	10	30			+	+	B		
	Formaldehyde	0.2%-8.0%	10	30			+	+	+	+	+
	Glutaraldehyde	2%	10	30			+	+	+	+	+
Gas	Ethylene Oxide	8-23g/ft3	60	60	37	30	+	+	+	+	+
	Paraformaldehyde	0.3 g/ft3	60	60	>23	60	+	+	+	+	+

NE= not effective
B = variable results dependent on virus
* available halogen (1:100)
**Princeton ,EHS

TABLE 3 - DISINFECTANT APPLICATIONS**

Disinfectants		Important Characteristics										
Type	Category	Work Surface	Dirty glassware	Large Area	Air Handling	Portable Equip Surface Decon	Portable Equip Penetrating Decon	Fixed. Equip Surface Decon	Fixed Equip Penetrating Decon	Optical & Electronic Inst	Liquid & Discard	Book Paper
Liquid	Quat. Ammon Cpds	+	+			+		+				
	Phenolic Cpds	+	+			+		+				
	Chlorine Cpds	+	+			+		+			+	
	Iodophor	+	+			+		+				
	Alcohol, Ethyl	+	+			+		+				
	Alcohol, Isopropyl	+	+			+		+				
	Formaldehyde	+	+			+		+				
	Glutaraldehyde											
Gas	Ethylene Oxide					+	+			+		+
	Paraformaldehyde			+	+	+	+		+	+		

**Princeton, EHS

APPENDIX A

Recommendations for Daily Preparation of Diluted Sodium Hypochlorite Bleach Disinfectant Solutions

The following published sources provide documentation regarding the need for daily preparation of fresh bleach solutions for disinfection purposes:

http://www.who.int/infectious-disease-news/IDdocs/whocds200317/8collection.pdf
The World Health Organization's *Guidelines for Collection of Specimens for Laboratory Testing* in the *Communicable Disease Toolkit, March 2003*, recommends daily preparation of bleach disinfectant solutions.
Chlorine solutions gradually lose strength, thus fresh solutions must be prepared daily. (See Annex 6, Page 10.)

http://www.cdc.gov/ncidod/dvrd/Spb/mnpages/vhfmanual/section5.htm
In its *Infection Control for Viral Hemorrhagic Fevers In the African Health Care Setting*, the Special Pathogens Branch of the CDC recommends the daily preparation of bleach disinfectant solutions. ***Bleach solutions must be prepared daily. They lose their strength after 24 hours. Anytime the odor of chlorine is not present, discard the solution.*** (See Section 5, *Disinfect Reusable Supplies and Equipment*.)

www.who.int/csr/resources/publications/surveillance/Annex7.pdf
In its *Guide for Field Operations, October 2006*, the World Health Organization (WHO) recommends the daily preparation of chlorine solutions. ***When preparing chlorine solutions for use note that chlorine solutions gradually lose strength, and freshly diluted solutions must therefore be prepared daily.*** (See page 39, Annex 7: *Disinfection*)

www.osha.gov/Publications/hand_hygiene.html
The U.S. Department of Labor publishes an OSHA *Quick Card* on Hand Hygiene that recommends the daily preparation of fresh bleach solutions for decontamination. ***Prepare fresh solutions daily, preferably just before use***. (See OSHA *Quick Card: Disinfecting Water for Tool/Surface Decontamination*)