

UNIVERSITY OF MARY WASHINGTON BSL-2 LABORATORY STANDARD OPERATING PROCEDURES (SOP)

This SOP document includes specific information for the organisms being used and procedures being performed.

All faculty, staff and students should familiarize themselves with these procedures and sign page 4 prior to starting work in this BSL-2 laboratory. Questions should be directed to the Principal Investigator. A copy of the SOP will be retained in the laboratory, by the Principal Investigator and by the Department Chair.

Principle Investigator: **Lynn O. Lewis**
BSL-2 Room Numbers: **Jepson 306, 324**
Biohazards Being Used:
Description of Procedure(s):

Hazards: The following materials and/or equipment associated with this procedure may present exposure hazards, health hazards, and/or physical hazards. Identify potential exposures that may occur during sample preparation, and/or experimental manipulations (i.e., use of sharps, aerosol generation during centrifugation, mixing or sonication, etc.):

- Cultures of organisms will be grown (utilizing incubator in Jepson 306 (Virology) only)
- Cultures will be stored in a sealed box inside the refrigerator in room 306, or in the -80°C freezer in room 321 (Instrument Lab)
- Cultures will be used to inoculate mice for either antibody production or infection (this is probably the biggest potential hazard – a sharp needle with an infectious agent)
- If centrifugation is performed, centrifuge tubes will be opened in the biological safety cabinet to minimize the risk of aerosol generation. Depending on speed needed, the centrifuge used with either be in room 321 or 322 (Tissue Culture)
- Any harvesting of blood or antibodies from infected mice will occur inside the biological safety cabinet

Administrative Controls: The following administrative controls are in place to avoid exposures (i.e., training, signage, restricted entry, etc.):

- Training will be conducted with all research students working in the lab
- Signs will be posted, alerting others to the presence of biohazardous material when procedures are being carried out
- Entry will be restricted while procedures are being carried out and while mice are infected and housed in room 306

Engineering Controls: The following safety equipment must be used when carrying out this procedure. (i.e., chemical fume hood, biological safety cabinet, sealed centrifuge rotors, etc.):

- Although not required for all procedures, any work being done with BSL-2 agents will be done inside the biological safety cabinet in room 306

Protective Equipment: The following personal protective equipment must be worn when performing this procedure (type of glove, eye protection, lab coat, etc.):

- Lab coats must be worn at all times, and left in room 306 when leaving the room
- Gloves must be worn at all times and disposed of in the Regulated Medical Waste bin
- Face shields will be available, but not required, unless working outside the biological safety cabinet (cleaning up a spill, for example)

Additional Special Handling Procedures: Including any transport between labs or buildings (i.e., secondary containment):

- There will be NO transport of materials (other than Regulated Medical Waste headed for treatment) between labs or buildings without the involvement of Dr. Lewis
- When Regulated Medical Waste is carried to the Autoclave Room (room 324), it will be sealed before leaving Room 306 and transported in an autoclave pan on a cart
- When mouse cages are ready for disinfection, the cages will be emptied into the Regulated Medical Waste bag (this should be done inside the biological safety cabinet). The cage will then be rinsed with 10% bleach (made fresh weekly) and wiped out. The cage will then be closed and transported to room 324 for autoclaving followed by washing in the glassware washer

Decontamination/Clean- Up Procedures: Specifics on products and procedures used to clean work areas. Include specifics on when these procedures will be performed and timing involved (i.e. contact time):

- Lab surfaces (bench tops, interior of biological safety cabinet) will be cleaned with either 70% ethanol or a 10% bleach solution (made fresh weekly) prior to use
- Lab surfaces (bench tops, interior of biological safety cabinet) will be cleaned with either 70% ethanol or a 10% bleach solution (made fresh weekly) after using the surfaces
- Any paper towels used to mop up the ethanol or bleach will be disposed of in the Regulated Medical Waste bin

Waste Disposal Procedures: Include specifics on collection, deactivation and transport for disposal:

- Any materials generated while working with BSL-2 agents will be considered Regulated Medical Waste and disposed of accordingly
 - This includes, but is not limited to culture plates, sharps, centrifuge tubes, pipettes, pipette tips, inoculated sacrificed mice
- Regulated Medical Waste can be collected in the bin (red bucket) for a maximum of 7 days – there will be a designated Waste Disposal Day every week (depending on sharps generation, sharps disposal may occur more or less frequently)
- Regulated Medical Waste will be placed in an orange autoclave bag inside the red bin as generated
- Regulated Medical Waste is treated by autoclaving at 135°C for 15 minutes prior to disposal (after cooling, the waste may be put in the regular trash, after labeling it as treated waste)
- Students will be trained in correct procedures for using the autoclave for treating Regulated Medical Waste, as well as record-keeping

Spill Response Procedures: Procedures to follow if a spill occurs:

- If a spill of a culture or contaminated material should occur, the following procedure must be followed:
 - ❖ Contact Dr. Lewis immediately
 - ❖ **For a spill inside the biological safety cabinet:**
 - LEAVE THE CABINET ON
 - Wear gloves, face shield and a disposable lab coat, and spray or wipe the cabinet walls, work surfaces and equipment with a 10% bleach solution
 - If necessary, flood the work surface, as well as drain pans and catch basins below the work surface, with 10% bleach solution for at least 20 minutes contact time
 - Soak up the disinfectant and spill with paper towels. Drain the catch basin into a container. Lift front exhaust grill and tray, and wipe all surfaces. Ensure that no paper towels or solid debris are blown into the area beneath the grill
 - Autoclave all clean up materials and protective clothing. Wash hands and exposed skin areas with disinfectant soap
 - If the spill overflows into the interior of the cabinet, the cabinet may need to be disinfected by a professional – contact Dr. Lewis
 - ❖ **For a small spill outside a biological safety cabinet:**
 - Wear gloves, face shield and a disposable lab coat, and cover the spill with paper towels and a 10% bleach solution for at least 20 minutes
 - Pick up the towels and discard into the Regulated Medical Waste bin

- Pick up any broken glass with forceps and place in a sharps container
- Re-wipe the spill area with bleach and wash hands (after removing gloves and discarding into the Regulated Medical Waste bin) with disinfectant soap
- ❖ **For a large spill outside a biological safety cabinet:**
 - GET HELP!!
 - The procedures are basically the same but on a larger scale

Injury/Exposure Response Procedures: Steps to be taken in the event of an exposure incident:

- Contact Dr. Lewis immediately
- Depending on exposure, medical assistance may be required
- Fill out an accident report with a copy to Dr. Lewis, the student and Dr. Dolby (department chair)

Unattended Operations: Portions of the experiment that may run unattended and steps taken to prevent accidental exposures:

- The only unattended portion of the experiment will be incubation of cultures in the incubator and incubation in mice
- Jepson 306 and 324 will be locked at all times and entry will be restricted during periods when mice are contained in the room (mice will be in special isolation cages)

I have read and understood all portions of this SOP. I agree to contact the Principle Investigator should I have any questions or plan on making any modifications to the procedures detailed here.

NAME	SIGNATURE	DATE
Lynn O. Lewis		

Standard UMW Biosafety Level 2 Practices and Procedures:

A. Standard Microbiological Practices

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.
2. Persons must wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are absolutely not permitted in the work areas. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.
4. Mouth pipetting is prohibited; mechanical pipetting devices are used.
5. Policies for the safe handling of sharps are instituted.
6. All procedures are performed carefully to minimize the creation of splashes or aerosols.
7. Work surfaces are decontaminated on completion of work or at the end of the day and after any spill or splash of viable material with disinfectants that are effective against the agents of concern.
8. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leakproof container and closed for transport from the laboratory. Materials to be decontaminated off-site from the facility are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.
9. An insect and rodent control program is in effect.

B. Special Practices

1. Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents is in progress. In general, persons who are at increased risk of acquiring infection, or for whom infection may have serious consequences, are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at increased risk of acquiring infections. The laboratory director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory or animal room.
2. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazards and meet specific entry requirements (e.g., immunization) may enter the laboratory.
3. A biohazard sign must be posted on the entrance to the laboratory when etiologic agents are in use. Appropriate information to be posted includes the agent(s) in use, the biosafety level, the required immunizations, the investigator's name and telephone number, any personal protective equipment that must be worn in the laboratory, and any procedures required for exiting the laboratory.
4. Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).
5. When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum

specimens may be collected periodically, depending on the agents handled or the function of the facility.

6. Biosafety procedures are incorporated into standard operating procedures or in a biosafety manual adopted or prepared specifically for the laboratory by the laboratory director. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

7. The laboratory director ensures that laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural or policy changes.

8. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.

a. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.

b. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

c. Syringes which re-sheath the needle, needleless systems, and other safety devices are used when appropriate.

d. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal, according to any local, state, or federal regulations.

9. Cultures, tissues, specimens of body fluids, or potentially infectious wastes are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.

10. Laboratory equipment and work surfaces should be decontaminated with an effective disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated according to any local, state, or federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.

11. Spills and accidents that result in overt exposures to infectious materials are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

12. Animals not involved in the work being performed are not permitted in the lab.

C. Safety Equipment (Primary Barriers)

1. Properly maintained biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are used whenever:
 - a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or embryonate eggs.
 - b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a biological safety cabinet.
2. Face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face when the microorganisms must be manipulated outside the BSC.
3. Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel.
4. Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate. Gloves are disposed of when overtly contaminated, and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Disposable gloves are not washed, reused, or used for touching "clean" surfaces (keyboards, telephones, etc.), and they should not be worn outside the lab. Alternatives to powdered latex gloves should be available. Hands are washed following removal of gloves.

D. Laboratory Facilities (Secondary Barriers)

1. Provide lockable doors for facilities that house restricted agents (as defined in 42 CFR 72.6).
2. Consider locating new laboratories away from public areas.
3. Each laboratory contains a sink for handwashing.
4. The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are inappropriate.
5. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.
6. Laboratory furniture is 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.
7. Install biological safety cabinets in such a manner that fluctuations of the room supply and exhaust air do not cause the biological safety cabinets to operate outside their parameters for containment. Locate biological safety cabinets away from doors, from windows that can be opened, from heavily traveled laboratory areas, and from other

potentially disruptive equipment so as to maintain the biological safety cabinets' air flow parameters for containment.

8. An eyewash station is readily available.

9. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

10. There are no specific ventilation requirements. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory. If the laboratory has windows that open to the exterior, they are fitted with fly screens.

* Adopted directly from *Biosafety in Microbiological and Biomedical Laboratories*, 4th Ed. U.S. Department of Health and Human Services, 1999.