

## UNIVERSITY OF NEVADA LAS VEGAS BSL-2 LABORATORY STANDARD OPERATING PROCEDURES (SOPS)

This SOP document should include specific information for the laboratories and procedures being performed. It is meant to give detail in addition to UNLV's adopted\* standard BSL-2 procedures (pages 6-9) and Exposure Control Plan (available through rms.unlv.edu).

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 must be forwarded to the UNLV Biosafety Officer and a copy must be retained in the laboratory's Biosafety Manual.

Principal Investigator: **PI Name, Title, Department**

BSL-2 Room Numbers: **Room Numbers**

Biohazards Being Used: **List Specific Organisms/Agents** (MSDS attached if available)

Description of Procedure(s):

**Include a description that is adequate enough so that the committee understands the process as well as what types of biological hazards exist.**

**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.):

**Study procedures with a potential for creating (accidental) aerosols or splashes include: handling of liquid cultures, centrifugation, vortexing, and streaking culture plates. These procedures will be performed in such a way as to minimize the potential for creation of splashes and aerosols. When removing vials and tubes from the centrifuge, the contents of the tubes will be allowed to settle before opening. These vials and tubes will be opened in a Class II Biological Safety Cabinet (BSC) with the mouth of the container pointing away from the face of the researcher. Health hazards associated with this BSL-2 agent and procedures are: accidental exposure through inhalation (e.g., splashes or aerosols), ingestion (e.g., touching nose/mouth with contaminated hands/gloves) or dermal contact with contaminated objects.**

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

**All staff are trained by senior laboratory personnel and by institutional Risk Management & Safety (RMS) staff. All personnel are required to complete "Chemical Hygiene" and "Biosafety" training provided by RMS. Biohazard signs are posted on all the laboratory doors indicating that it is a Biosafety Level 2 (BSL-2) laboratory, the supervisor's name (or other responsible personnel), and contact telephone number(s). Entrance to the laboratories is restricted to authorized personnel only by means of a lockable door with limited keys. In addition, after hours the BSL-2 samples are stored in a locked refrigerator.**

**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.): **Handling contaminated materials takes place in the BSC. The BSC is certified annually by an outside contractor approved by UNLV. All centrifugation steps are performed with plastic screw-cap centrifuge tubes. Stock cultures are stored separately from reagents in a refrigerator and/or an ultra-low freezer.**

**Protective Equipment:** The following personal protective equipment must be worn when performing this procedure (type of glove, eye protection, lab coat, etc.): **Personnel are required to wear disposable gloves and lab coats while conducting experimentation in the laboratory. Lab coats have assigned users and will remain in the laboratory at all times. Lab coats must either be discarded in biohazard waste or laundered by laundry service approved by Risk Management & Safety. Gloves must be discarded after use, if soiled or compromised, or before exiting the laboratory. Gloves are not to be reused and are to be disposed with the biological waste in a biohazard bag located in the laboratory. Safety glasses or prescription safety glasses will be worn when conducting experimentation. Personnel must wash their hands after handling contaminated materials and removing gloves, and before exiting the laboratory.**

**Additional Special Handling Procedures:** Including any transport between labs or buildings (i.e., secondary containment): **For transporting, biological material between laboratories within the building, the biological material will be placed in an enclosed, labeled, primary culture tube or plate. The primary tube or culture plate will be placed in a secondary container (e.g. a sealable biohazard plastic bag). For transporting outside the building, the secondary container will be placed into a rigid sturdy outer container that has a lid that snaps tight to prevent it from opening. Following transport of biological materials between laboratories or outside the building, the secondary container and primary culture tube or plate are disinfected as indicated below in the decontamination/clean-up procedure.**

**In addition, biological culture tubes will be transported from \_\_\_\_ hospital to UNLV's room \_\_\_\_\_. All primary culture tubes that will be picked up will be individually labeled according to their contents and lids will be sealed with Para film. The primary culture tubes will be placed into a secondary bag that has a biohazard symbol on it with absorbent material. The secondary bag will be placed into a cooler that has a lid that snaps that prevents it from opening. Leak proof ice packs will be placed inside the cooler as well. A DOT Class 6.2 Infectious Substance and Biohazard label will be placed on the outside of the cooler. A bill of lading, Material Safety Data Sheet and an emergency contact sheet which includes the biology departments information (address, phone number, and contact person) will be placed on the outside of the cooler in a pouch. The Principal Investigator/Supervisor transporting the materials will accompany the materials at all times. Anyone transporting the biological material will have current DOT 6.2 Infectious Substance Training provided by RMS before transporting the biological culture material. Note: The PI/Supervisor can designate an identified project assistant to transport the materials provided the designee has current DOT Class 6.2 Infectious Substance training.**

**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):

**For work conducted in the BSC, all materials and labware will be disinfected with a 1% sodium hypochlorite solution for \_\_\_\_ minutes before removal from the BSC. Laboratory benches will be sprayed with 1% sodium hypochlorite and wiped with paper towels before and after each experiment. Contaminated paper towels will be discarded in a biohazard bag located in the laboratory.**

**The BSC will be disinfected with a 1% sodium hypochlorite solution and followed up with 70% ethanol. All reusable glassware will be decontaminated by steam sterilization (i.e., autoclaving) for at least 30 min. at 121 degrees Celsius and 15 psi. All disposable labware contacting biological materials (e.g., serological pipettes, Petri dishes) must be autoclaved prior to disposal. Following sterilization, reusable glassware will be rinsed and scrubbed, and either hand washed or machine washed with soap and water.**

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

**Biological waste will be consolidated at the end of each day, or as needed, and placed in a large biohazard bag near the autoclave. All biohazard bags will be double-bagged when 1/2 full, or as needed (not less than weekly), placed in a leak-proof plastic bin, and autoclaved for 1 hr. at 121 degrees Celsius and 15 psi. A biological indicator strip will be included with each autoclave run to corroborate the efficacy of the sterilization. Upon completion of sterilization, biological waste will be stored until picked up by the UNLV Biohazard Waste Contractor. Disposable glassware (e.g., screw-capped tubes for biological indicator strips) are autoclaved as indicated above, rinsed, and disposed in the broken glass (plastic lined) box until full. Contaminated sharps are disposed of in the sharps containers that remain inside the BSCs. Sharps containers are bagged, taped, and autoclaved when full. Autoclaved sharps containers are disposed of by the UNLV Student Health Center.**

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

**Spills involving biological material will be treated with 1% sodium hypochlorite, allowed the appropriate contact time, and contained with absorbent material or paper towels. All personnel in the immediate area will be notified at once and asked to leave the area. Contaminated clothing will be turned inward, and placed in a biohazard bag. The Principal Investigator/Supervisor will be informed and no one will re-enter the room until the Principal Investigator/Supervisor permits it. Before re-entering the room to proceed with the clean-up, appropriate personal protective equipment will be used such as: gowns, gloves, shoe covers, and a respirator with a N95 cartridge will be worn - individuals wearing respirators will be FIT tested by RMS. Starting at the edge and working toward the center of the spill, paper towels or absorbent material will be placed on the spill. 1% sodium hypochlorite will be poured over the paper towels and starting around the edges and working toward the center saturating the area. Allow sufficient contact time (\_\_\_\_ minutes - stated in the MSDS). All contaminated absorbent materials will be disposed of in a double, sealed biohazard bag, and autoclaved as indicated above (see Waste Disposal**

Procedures). Biological spill kits are available in the "Spill Kit Area" located

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**Injury/Exposure Response Procedures:** Steps to be taken in the event of an exposure incident: In case of injury or exposure of laboratory personnel to viable microbiological material, the affected bodily area will be washed with abundant mild soap and water. If the contamination is in the eyes, eye wash stations will be used to flush the contaminant. If necessary, RMS will be notified, and an Incident Report will be filed with the Insurance Administrator. At the discretion of the exposed person and/or the Principal Investigator, the exposed individual may decide to report to an urgent care facility for evaluation and possible treatment. Laboratory personnel are required to report to the Principal Investigator any unusual symptoms that they experience as a consequence of the injury/exposure.

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

Inoculated cultures (Petri dishes and broth) will be incubated unattended. All incubations will be done inside sealed incubators and rotary shakers with an affixed Biohazard Sticker.

**Additional Laboratory Specific Safety Procedures:**  
(Refer to attached MSDS)

1. Long hair should be tied back when working with or near an open flame.
2. Make-up cannot be applied in the laboratory (this includes lip balm).
3. Contact lenses can be worn in the laboratory as long as the user wears safety glasses.
4. Open-toe shoes cannot be worn in the laboratory.
5. Lock-up of the laboratory at the end of the day requires a walk-through that involves verifying the following:
  - a.) all propane torches or bunsen burners are in the off position,
  - b.) water baths are turned off,
  - c.) hot plates are turned off,
  - d.) other equipment (not in use) such as rotary shakers and centrifuges are turned off, and
  - e.) laboratory doors are locked

**Variations from UNLV Standard Biosafety Level 2 Practices and Procedures and Reasons:**

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

NAME	SIGNATURE	DATE

## Standard UNLV Biosafety Level 2 Practices and Procedures:

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

### **A. Standard Microbiological Practices**

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:
  - a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
  - b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
  - c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
  - d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:
  - a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
  - b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include: the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and

required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.

10. An effective integrated pest management program is required.

11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

### **B. *Special Practices***

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

2. Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.

3. Each institution must establish policies and procedures describing the collection and storage of serum samples from at-risk personnel.

4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.

5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.

6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.

7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.

a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.

b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.

8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety safety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.

9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.

10. All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.

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

1. Properly maintained BSCs (preferably Class II), other appropriate personal protective equipment, or other physical containment devices must be used whenever:

a. Procedures with a potential for creating infectious aerosols or splashes are conducted.

These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.

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

**D. Laboratory Facilities (Secondary Barriers)**

1. Laboratory doors should be self-closing and have locks in accordance with the institutional policies.
2. Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.
3. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
  - a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
  - b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
5. Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.
6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
8. An eyewash station must be readily available.

9. There are no specific requirements on ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.

10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.

11. A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

\* Adopted directly from *Biosafety in Microbiological and Biomedical Laboratories*, 5<sup>th</sup> Ed. U.S. Department of Health and Human Services, 2007.