**Guidance from the Wayne State University Institutional Biosafety Committee (IBC) regarding preparation of Biosafety Laboratory Standard Operating Procedures (SOP)**

SOPs should be specific to the procedures carried out in your lab and should be considered as a framework for development of laboratory- and procedure-specific protocols that relate to safe execution of the intended experiments. The primary audience is those who will do the work. In addition to being an operational reference for experienced personnel, the SOP should be useful as part of a training plan for new personnel. SOPs should be understandable without reference to external documents such as animal use protocols, grant applications, etc. Ideally, biosafety information should be incorporated into procedural / operational laboratory SOPs, but you may use any format if each of the categories listed below are addressed. SOPs should be free of spelling and grammatical errors.

Even if you propose the use of seemingly safe systems, carefully describe the potential risks and how you intend to mitigate them. If you are using a replication-incompetent or otherwise attenuated biological vector system, explain the basis of the safety-related features of the system.

If animals are exposed to biological agents and may pose a hazard to DLAR personnel, complete the in vivo section SOP. If a section is not applicable, indicate N/A. The PI is responsible for informing DLAR staff of the biosafety procedures outlined on this SOP prior to exposing animals to biological agents. All affected lab staff should read and sign the SOP.

**1. Hazards:**

Describe specific procedures, materials and/or equipment associated with this protocol that may represent exposure and/or health hazards (e.g., mixing, centrifuging, sonication, or needle use may produce aerosols), and identify all potential routes of exposure (i.e., inhalation, ingestion, injection, mucous membrane/skin contact) that may occur during these procedures.

Do not minimize the potential hazards associated with the handling of a BSL2 agent; *defensive statements explaining that hazards are minimal are not an acceptable description of the hazards.*

The subsequent sections of the SOP must identify the methods used to mitigate the risks identified that are either agent specific, or procedure specific.

**Hazards, DLAR staff:** Address the possibility that virus / bacteria / toxin may be present in the animal cages/bedding.

**2. Engineering Controls:**

List each procedure that may represent an exposure hazard, and the engineered safety / containment equipment to be used when carrying out these procedures (e.g., microisolator cages, biosafety cabinet, downdraft table, filter dumping station, etc.). Avoid reference to a "laminar flow hood"; please specify if a chemical fume hood (CFH) or biological safety cabinet (BSC) will be used.

If centrifugation is involved, describe how aerosols will be contained when tubes are opened and how centrifuges and rotors will be disinfected after use. Centrifuges should have sealed rotors, gaskets and/or safety cups, and only be opened inside a biosafety cabinet for loading and unloading of samples.

Centrifuge Safety Tips - Loading and unloading a centrifuge tube holder or centrifuge rotor:

1. Fill specimen tubes/vials containing biological agents inside a biosafety cabinet.
2. Cover tubes/vials with cap/lid/parafilm.
3. Place tubes/vials into centrifuge tube holders (bucket) or rotor (inside biosafety cabinet, if possible).
4. Place centrifuge bucket covers/safety cups on tube holders, if available, or screw down rotor cover.
5. After centrifugation, return tube holders (with bucket covers/safety cups still in place) or covered rotor to biosafety cabinet to unload tubes/vials, ***or*** remove the capped/covered tube and open inside a biosafety cabinet.

WSU’s IBC discourages the use of open flames to sterilize and/or reduce cross contamination inside a BSC. Please see the [Gas Use in Biosafety Cabinets](https://research.wayne.edu/oehs/pdf/gas-in-bsc.pdf) fact sheet for additional information.

**3. Personal Protective Equipment (PPE):**

List PPE required when performing listed procedures, entering rooms and/or handling infected animals. Include a statement that describes when the PPE is required. This should always include a lab coat or dedicated “scrub” uniform, gloves, and eye protection. Specify glove type; if latex gloves are specified, also specify an alternative material (e.g., Nitrile).

Generally, NIOSH-certified respiratory equipment (e.g., N95) should only be required for work if the engineering controls (biological safety cabinet or chemical fume hood) are not sufficient to prevent exposure to potential airborne aerosols. If a respirator is to be used, please specify the type (N95, etc.). Surgical masks and disposable face masks that are not certified by NIOSH are primarily designed to provide protection from contamination from the wearer (cough, etc.) and not considered to be personal protective equipment. A N95 respirator is suggested for personnel entering an animal room.

Please see: <http://research.wayne.edu/oehs/health-safety/respirators.php> for MIOSHA requirements under the Respiratory Protection Standard (including training, medical qualification, and fit testing).

**4. Additional Special Handling Procedures:**

Describe how agents/animals will be transported from one lab or building to another (using secondary containment), and correct labeling requirements (i.e., Outer container must be labeled with the name of biological agent, name of PI, contact information, and labeled with a biohazard symbol), or any other safety considerations related to the research that is not included elsewhere in the SOP.

If a needle and syringe will be used, please stated how needlestick injuries will be prevented (saying "utmost care will be taken" is not sufficient). For guidance for safe injection techniques, please see: <http://research.wayne.edu/oehs/anicon/safe-animal-injections.doc>

**5. Decontamination / Clean up:**

Identify products and procedures to clean work areas, cabinets, etc. daily when that day’s work is completed. Indicate the product name, manufacturer, and working concentrations or dilutions (e.g., fresh 10% bleach) plus the required contact time for any commercial disinfectants you will use. A detailed description of the decontamination/clean-up procedures should be provided rather than referring the reader to another place in the document.

Bleach is an effective disinfectant for non-metal surfaces. Bleach, even diluted with water, will pit, and corrode stainless steel surfaces of biosafety cabinets, animal cages, or other equipment with stainless steel surfaces, making disinfection more difficult and shortening the equipment’s life expectancy. Due to bleach’s corrosive properties, use a suitable non-bleach disinfectant to decontaminate biological safety cabinets, centrifuge rotors, and other corrodible surfaces. If a 10% bleach solution must be used on a biological safety cabinet or other stainless-steel equipment, a final rinse using sterile water should be applied to remove the chlorine residue from the surface, preventing corrosion.

70% ethanol (or a similar alcohol-based cleaner) is not recommended for surface decontamination for potentially infectious agents. These types of disinfectants evaporate too quickly to provide sufficient surface contact time to kill microorganisms. Alcohol-based cleaners can be used as a final step to clean a surface (after proper disinfectant use/ contact time) to remove any residue left behind. Wescodyne © (iodine-based solution) can be used as an alternative. It should be diluted 1:213 (0.6 ounces of Wescodyne per gallon of cold water) when used to decontaminate biosafety cabinet and other stainless-steel surfaces. Surface contact time of 15-20 minutes is needed to for proper decontamination. Other disinfectants can also be used, including phenolics or quaternary ammonias. Refer to manufacturer data to determine efficacy against the biological agent you will be working with.

The NIH does not recommend or support the use of ultraviolet (UV) radiation in laboratories. UV has limited penetrating power and is primarily effective against unprotected microbes on exposed surfaces or in the air. It does not penetrate soil or dust. The intensity or destructive power decreases quickly with distance from the lamp. The intensity of the lamp diminishes over time and is drastically affected by the accumulation of dust and dirt on it. The bulbs require frequent maintenance. Biosafety cabinets must be decontaminated prior to maintaining their UV lights. In addition, the use of UV requires PPE or other controls to protect users. Good techniques in conducting experiments are highly effective in preventing contamination, and the use of UV radiation does not eliminate the necessity for using good practices and procedures.

**6. Waste Disposal:**

Describe how you will collect and treat liquid and solid biological waste, and sharps.

**Liquid Biohazardous Waste Treatment and Disposal:** To treat liquid biological waste, make a final dilution of 1:10 with bleach and let sit for at least 30 minutes. Add bleach to your collection flask/ container as you go along, as bleach will degrade over time and lose its disinfecting quality. After the liquid is treated and allowed to sit, it is no longer a biological waste, and it can be flushed down the drain with copious amounts of water. If possible, use a separate sink for hand washing and treated liquid waste disposal. Wescodyne- (iodophor) treated biological liquid waste should not be disposed of down the drain and must be collected for disposal as a chemical waste.

**Solid Biohazardous Waste Treatment and Disposal:** Place material in an autoclave bag - the biohazard bins and liner bags provided by OEH&S will melt in an autoclave. Autoclave bag holders may be placed near workstations where waste is generated and can be ordered from lab safety supply catalogs like Fisher Scientific or Lab Safety Supply. The holders should be hard sided, leak proof, puncture resistant containers with a lid. Alternatively, OEHS can provide M64 mediwaste containers. These are suitable for BSL-2 facilities, but a foot pedal stand must be purchased from the vendor (Daniels). Waste does not need to be autoclaved on-site. OEHS has a contract with a vendor (Daniels) for the processing of biologically hazardous solid waste.

Remember: do not place treated waste (containing bleach or other chemicals) in a biohazardous waste container. Do not overfill red bins! Make sure they can be closed securely and weigh less than 30 lbs. When finished, tie the red bin liner closed, and request a pickup using OEHS’s on-line request form: <http://research.wayne.edu/oehs/forms/bio-waste.php>

**Sharps Disposal:** Describe how sharps will be disposed. Sharps should be directly deposited into sharps container; avoid re-capping needles.

**Lab glassware and plasticware** that is NOT contaminated with hazardous chemicals, human or animal blood, or any other potentially infectious material should be disposed of in “sturdy” cardboard boxes lined with a plastic liner. Materials (e.g., pipettes, flasks, etc.) may be disinfected with a bleach solution or other appropriate disinfectant. Boxes should not weigh more than 25 pounds. When full, tape shut and label the box “glass waste” for collection by custodial staff.

**Waste Disposal Procedures, DLAR Staff**: Bedding and waste from infected animals should be treated as biohazardous, collected, and autoclaved per standard DLAR SOPs. If there is any deviation from the standard procedures used in the DLAR, and as outlined in the *in vivo* SOP, they must be described in the relevant sections.

**7. Spill response procedure:**

Describe procedures to follow if a spill occurs in the biosafety cabinet, on the bench, etc. E.g., assess risks - call OEHS if large spill; don PPE, cover spill with absorbent material, pour liquid disinfectant on spill, let sit for required contact time, clean up and dispose in appropriate waste container (e.g., Biohazardous or Chemical Waste). Please note that disinfectant saturated absorbent material must be collected for disposal as chemical waste.

**Spill Procedures: If inside a BSC or CFH, spill clean-up can commence immediately. If the spill occurs outside of containment, all personnel should vacate the immediate vicinity for 15 minutes to allow aerosols to settler prior to commencing the spill clean-up procedure.**

1. Wear appropriate PPE (provide details based on PPE section above).
2. Place paper towel over spill to help contain the material and prevent it from spreading on surface/floor.
3. Carefully pour (or spray) liquid disinfectant on top of paper towel, starting on the outside and moving towards the center in concentric circles *(Provide specific name of disinfectant).*
4. Let stand for at least X minutes *(Provide contact time required for specific disinfectant in use. If using a 10% dilution of bleach, the contact time is 30 minutes)*. This enables the disinfectant to decontaminate the surface.
5. Collect all the disinfectant saturated materials as Chemical Waste. Dispose of all other potentially contaminated items as Biohazardous Waste.
6. Clean the surface with soapy water or 70% alcohol.

If you suspect a tube has broken during centrifugation and its contents have spun out, follow these steps:

1. Turn off and unplug the centrifuge and do not open the lid for at least 30 minutes.
2. Don PPE, including gloves, lab coat, eye protection, and N95 respirator (optional).
3. After 30 minutes, open the lid and remove the contaminated buckets and rotor.
4. Clean the buckets and rotor in a biosafety cabinet with a suitable disinfectant that will not damage or corrode the steel or rubber O-rings/ gaskets. Bleach is not recommended due to its corrosive properties.
5. Remove any broken glass or plastic from centrifuge with forceps and place in sharps container.
6. Clean out the inside of the lid and centrifuge with a suitable disinfectant (one that will not damage or corrode the parts). Remember that contact time is important when disinfecting contaminated surfaces (15-20 minutes minimum).
7. Dispose of paper towels in appropriate waste container; do not autoclave materials that are contaminated with bleach or other disinfectant chemicals.

**8. Injury/Exposure Response:**

List the steps to be taken in the event of an exposure incident. To complete this section, please fill in the specific information about your lab and the agent in use. List available antibiotic(s) or prophylactic treatment(s) to be used in case of exposure. (If none, state "none" - do not leave blank).

Please provide symptoms of infection associated with the agents in use.

For More Information:

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