



## Standard Operating Procedure Biosafety Level 2 (BSL-2)

### Standard Laboratory Practices

- Access to the laboratory is limited or restricted at the discretion of the laboratory director.
- Placards should be placed on the entrances to the lab listing biological hazards and the PI's name and 24/7 contact information for the PI and/or laboratory personnel familiar with the biohazard.
- Do not store food in lab.
- Do not eat, drink, smoke, handle contact lenses, apply cosmetics (including chap stick), etc. in the lab.
- Do not mouth pipette!
- Plants or animals not involved in experiments are not allowed in the lab.
- Laboratory personnel must be appropriately trained.
- The safety protocol (SOP) serves as training documentation and reference information. A copy signed by laboratory personnel should be stored in the lab's safety manual.
- Vacuum lines must be HEPA filtered or second flask.
- Liquids should be handled carefully to minimize creation of splashes and aerosols. Centrifugation should be performed using sealed tubes and sealed rotors or safety cups.
- All procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.
- Sharps should be handled with extreme caution to avoid cuts or autoinoculation during use and disposal. Needles should not be bent, sheared, or recapped. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, by autoclaving or incineration.
- Transport: Infectious or biohazardous materials must be transported in a sealed primary container inside a sealed durable and leak proof secondary container that has been labeled with a biohazard sticker
- Laboratories must have a sink for handwashing. Lab personnel must wash their hands after they handle viable materials and animals, after removing gloves, and before leaving the laboratory or animal facility.
- An eyewash station must be readily available.
- Laboratories should be designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.
- Laboratory furniture must be non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
- A method for decontaminating all laboratory wastes should be available in the facility (e.g. autoclave, chemical disinfection, or other validated decontamination method).
- BSCs must be installed in such a manner that fluctuations of the room supply and exhaust air do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other potentially disruptive equipment.
- Risk Group 2 agents shall be handled with appropriate precautions consisting primarily of good microbiological laboratory techniques as well as Biosafety Level 2 (BSL-2) containment.

### Personal Protective Equipment (PPE)

- Wear protective gear including disposable gloves and a cloth or disposable lab coat. If using a cloth lab coat, it must remain in the cell culture room, virus procedure room or inside a biohazard bag in the lab.
- Wear safety glasses and face protection when splashes, sprays or aerosols can be expected.
- Dispose of contaminated PPE in biohazard bags/containers.
- No personal protective equipment shall be worn outside of the lab.

## Working Inside the Biosafety Cabinet (BSC)

- Hands and arms should be washed well with germicidal soap before and after work in the BSC.
- Technicians are encouraged to wear long sleeve gowns with knit cuffs and latex or nitrile gloves. This minimizes the hands and arms from contamination by infectious agents.
- Interior surfaces of the work area should be disinfected by wiping them thoroughly with 70% alcohol.
- Everything needed for the complete procedures should be placed in the BSC before starting so that nothing passes in or out through the air barrier until the procedure is completed.
- Do not place anything over the front intake or rear exhaust grill in units having a solid work surface. Keep equipment at least four inches inside the cabinet window and perform transfer of viable materials as deeply into the BSC as possible.
- After all materials have been placed in the BSC, wait 2-3 minutes before beginning work.
- Minimize room activity which can create disruptive air current.
- Minimize the movement of objects (including hand and arms) into and out the BSC.
- The BSC should not be overloaded.
- Lab contamination control procedures and aseptic techniques are necessary while working in the BSC.
- Do not use a Bunsen burner in a BSC.
- Do not use the BSC to store excess laboratory equipment.
- Following completion of work, the following steps must be performed
  - Allow the BSC to run 2-3 minutes with no activity.
  - Decontaminate the interior surface with 70% alcohol.
  - Shut down by turning off the fan and lights.

For more detailed information regarding how to use BSC, please check Laboratory Safety Manual section 8.

## Working Procedures for Viral Vectors

- **Before working with virus**
  - Prepare a solution of 10% bleach in water in appropriate containers for disinfecting supplies that may come in contact with the virus. This solution should at minimum be prepared fresh weekly. Alternatively, use Virkon, NecPhene or VesPhene.
  - Put on PPE
- **While working with virus**
  - Always use aseptic technique
  - Avoid the spread of contamination and immediately replace gloves, if contamination is suspected.
- **After working with virus**
  - Treat liquid waste with bleach to a final concentration of 10% bleach for a minimum contact time of 30 minutes. After 30 minutes, dispose of liquid waste in a sink with copious amounts of running water.
  - Solid waste including pipettes, containers, etc. that come in contact with virus must be disinfected with 10% bleach prior to disposal in biohazard waste container. Autoclave biohazard waste upon completion and place it in black bags for final disposal.
  - Disinfect work surfaces with 70% ethanol.
  - Remove disposable PPE and dispose of it in biohazard bags.
  - Wash hands with soap and water.

## **Spill Procedures**

- Alert personnel in vicinity to leave the immediate area.
- Don protective equipment (gown/lab coat, gloves, eye protection).
- Cover an area twice the size of the spill with paper towels, or other absorbent material.
- Pour disinfectant solution onto the spill, starting at the perimeter and working inward from the edges of the towels. Avoid splashing.
- Allow 30 minute contact period.
- Wipe down any contaminated stationary equipment or furniture twice with disinfectant. Contaminated fabric-covered furniture or porous material should generally be treated with disinfectant.
- Use forceps, tongs, or broom to remove broken glass and other items; place in sharps container or red bag, as appropriate.
- Remove towels and re-clean area with disinfectant solution.
- Collect and dispose in autoclavable biohazard bags.
- Decontaminate (autoclave, or use a chemical disinfectant) reusable clean-up items and other permanent equipment.
- Inform laboratory personnel when the clean-up is complete.
- Dispose of contaminated PPE in autoclavable biohazard bags.

## **Spills inside a Biological Safety Cabinet**

- Keep the cabinet running.  
Clean-up as per directions above, making sure to wipe down back and side walls of cabinet.
- If material has spilled into the catch basin beneath the work surface, add a volume of disinfectant equal to the quantity in the basin, wait 20 minutes, and absorb with paper towels.
- After completion, allow cabinet to run for ten minutes before resuming work.

## **Spills inside a centrifuge**

- Shut centrifuge off and do not open the lid for 20 minutes to allow aerosols to settle.
- Put on PPE.
- Use a squeeze bottle to apply disinfectant to all contaminated surfaces within the chamber, taking care to minimize splashing.
- Allow 20 minute contact period and then complete clean-up of the chamber.
- Remove buckets and rotors to nearest Biological Safety Cabinet; disinfect and clean as per manufacturer's instructions.