

 <p>GALLOGLY COLLEGE OF ENGINEERING BIOPROCESSING CORE FACILITY <i>The UNIVERSITY of OKLAHOMA</i></p>	<p>Title: BSL-2 Laboratory Safety Guidelines for OU Bioprocessing Core Facility Users</p>		
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1. PURPOSE

1.1. The purpose of this procedure is to describe the use of basic aseptic techniques to enhance safety at OU Bioprocessing Core Facility (OUBCF) by ensuring that everyone working in a biosafety cabinet is aware of the measures that are in place to protect them and avoid potential exposure to biohazardous materials.

2. POLICY

2.1. All analysts/students must read this procedure before entering the OUBCF and conducting any experiments.

3. RESPONSIBILITY

3.1. It is the responsibility of each student/analyst working at OUBCF to follow this procedure when working inside a BSC and on the bench-top when executing applicable testing/experiment.

4. SAFETY

- 4.1. All applicable safety procedures within the OUBCF will be followed per guidelines.
- 4.2. Human tissue and blood products are potentially infectious and must be handled according to OSHA regulations and universal precautions for preventing transmission of blood-borne pathogens. Liquid aerosol and physical contact with human tissue, fluids and blood products are potentially hazardous for infection from HIV and hepatitis.

5. DEFINITIONS

- 5.1. **IsoPropyl Alcohol (IPA):** A general cleaning solvent that is used to clean and disinfect various substances/substrates in the lab.
- 5.2. **Aseptic Technique** – Preventive measures taken to prevent microbial contamination of the personnel, the environment they are working in or contamination of the specimen they are working on.
- 5.3. **BSC** – Biological Safety Cabinet – A piece of equipment that is specially designed to provide a microbe-free work area. A BSC draws air through the front of the working area into the vent system; it is then recirculated and expelled.
- 5.4. **Disinfected item** – An item that is sterile inside its packaging, but the outside has been disinfected with an approved disinfectant (e.g. 70% IPA). The outside of the item is not sterile.
- 5.5. **PPE** – Personal Protective Equipment – materials worn to protect individuals from exposure to chemicals or materials (e.g. safety glasses, laboratory coats).
- 5.6. **Sterile**– The state of being free of viable microorganisms/biological agents.

5.7. **OSHA**—The Occupational Safety and Health Administration is a regulatory agency of the United States Department of Labor that assures safe and healthful working conditions by setting and enforcing standards, and by providing training, outreach, education and assistance.

6. EQUIPMENT AND MATERIALS

- 6.1. BSC
- 6.2. Sterile Alcohol, 70%
- 6.3. Paper towel and Kim-wipes
- 6.4. 3. Biohazard bag
- 6.5. Discard pipette tray or box

7. STANDARD LABORATORY PRACTICE

- 7.1. Access to the laboratory is limited or restricted at the discretion of the laboratory director or scientist.
- 7.2. Laboratory personnel must be provided with medical surveillance as needed and offered available immunizations for agents handled or potentially present in the laboratory.
- 7.3. Do not store food in the lab.
- 7.4. Do not eat, drink, smoke, handle contact lenses, apply cosmetics (including Chap Stick), etc. in the lab.
- 7.5. Do not mouth pipette!
- 7.6. Liquids should be handled carefully to minimize the creation of splashes and aerosols.
- 7.7. Infectious agents may be centrifuged in the open laboratory using sealed rotor head or centrifuge safety cups.
- 7.8. All procedures in which infectious aerosols or splashes may be created must be conducted in BSCs or other physical containment equipment.
- 7.9. 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, which is placed into a biohazard box that is marked for incineration.
- 7.10. All infectious or biohazardous materials must be transported in a sealed primary container inside a sealed durable and leak proof secondary container labeled with a biohazard sticker.
- 7.11. Lab personnel must wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory or facility.
- 7.12. An eyewash station must be readily available.
- 7.13. All laboratory wastes such as cultures, stocks, and other potentially infectious materials before disposal should be decontaminated using the available methods in the Lab. e.g. autoclave, chemical disinfection, or other validated methods.
- 7.14. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leak proof container with a biohazard label and secured for transport.

7.15. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

8. PERSONAL PROTECTIVE EQUIPMENT (PPE)

- 8.1. Basic aseptic technique requires that analysts and students must be constantly aware of their own actions and surroundings to ensure sterility during the execution of all practices inside the BSC and on the laboratory bench-top.
- 8.2. Protective laboratory coats, gowns, gloves, safety glasses or corrective eyeglasses designated for laboratory use must be worn while working with hazardous materials.
- 8.3. Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays or infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device.
- 8.4. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
- 8.5. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

9. PROCEDURE

- 9.1. Hands and arms should be washed well with germicidal soap before and after work in the BSC.
- 9.2. Before entering the BSC don sterile gloves. Spray gloves with 70% IPA and then don sterile sleeve covers, if applicable. Spray hands with 70% IPA prior to entering the BSC.
- 9.3. The interior surfaces of the work area should be disinfected by wiping them thoroughly with 70% alcohol. While cleaning the BSC, make sure to remove all materials in order to clean all surfaces of the BSC.
- 9.4. Keep equipment at least four inches inside the cabinet window and perform transfer of viable materials as deeply into the BSC as possible.
- 9.5. After all materials have been placed in the BSC, wait 2-3 minutes before beginning work. Do not touch hair, face, safety glasses, laboratory coat or any other item outside the BSC with gloved hands. If any items touch gloves, replace gloves and spray them with 70% IPA prior to reentry to the BSC.
- 9.6. While working under the BSC ensure to make slow deliberate movements so that proper airflow is maintained. Do not make any quick movements.
- 9.7. Do not crowd the BSC with materials. Keep materials away from the return air grills to ensure proper airflow.
- 9.8. Lab contamination control procedures and aseptic techniques are necessary while working in the BSC and when passing items into the BSC.
- 9.9. Inspect all items for tears, holes, and seal integrity prior to being passed into the BSC. Any item found with a defect could be sterility compromised. Discard and replace compromised items immediately.
- 9.10. Do not use the BSC to store excess laboratory equipment.
- 9.11. When introducing double wrapped items to the BSC, pass the items in such a way as to ensure to remove the outer wrapping so that it does not contact the inner item.
- 9.12. In order to reduce the risk of possible contamination the operator once working under the BSC

should minimize the number of times that one must go in and out of the BSC.

- 9.13. Arrange the items in the BSC in a logical pattern from clean to dirty to avoid passing contaminated materials through airflow above clean materials.
- 9.14. Keep bottles, jars, tubes capped until ready to use. Gently lift the cap, without touching the bottle, jar, or tube insert pipet and carefully draw up or dispense solution. Remove pipet without touching the bottle, jar, or tube and then cap the containers.
- 9.15. Do not handle chairs, footstools, or other items with your hands. Push or pull the item with your foot.
- 9.16. Once all work inside the BSC is complete and the operator exits the BSC gloves should be discarded when possible.
- 9.17. Following the completion of work, the following steps must be performed:
 - 9.17.1. Allow the BSC to run for 2-3 minutes with no activity.
 - 9.17.2. Decontaminate the interior surface with 70% alcohol.
 - 9.17.3. Shut down by turning off the fan and lights.

10. SPILL PROCEDURE

- 10.1. Alert personnel in vicinity to leave the immediate area.
- 10.2. Don protective equipment (gown/lab coat, gloves, and eye protection as needed).
- 10.3. Cover an area twice the size of the spill with paper towels, or spill kit, if available.
- 10.4. Pour freshly prepared 10% bleach solution onto the spill, starting at the perimeter and working inward from the edges of the towels. Avoid splashing.
- 10.5. Allow a 10-minute contact period. Contact time should be appropriate for the disinfecting agent used in the lab. See manufacturer's instructions.
- 10.6. Wipe down any contaminated stationary equipment or furniture twice with disinfectant. Contaminated fabric-covered furniture or porous material should generally be treated with disinfectant.
- 10.7. Use forceps, tongs, or broom to remove broken glass and other items, place in sharps container or red bag, as appropriate.
- 10.8. Remove towels and re-clean area with disinfectant solution.
- 10.9. Collect and dispose in autoclavable biohazard bags.
- 10.10. Decontaminate (autoclave or use a chemical disinfectant) reusable clean-up items and other permanent equipment.
- 10.11. Inform laboratory personnel when the clean-up is complete.
- 10.12. Dispose of contaminated PPE in autoclavable biohazard bags.
- 10.13. For spills inside a BSC, perform the following steps:
 - 10.13.1. Keep the cabinet running.
 - 10.13.2. Clean-up as per directions above, making sure to wipe down back and side walls of cabinet.

- 10.13.3. 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 10 minutes, and absorb with paper towels.
- 10.13.4. After completion, clean the BSC with sterile water to remove all the bleach residue. Then, allow the cabinet to run for ten minutes before resuming work.
- 10.14. For spills inside a centrifuge, perform the following steps:
 - 10.14.1. Shut centrifuge off and do not open the lid for 30 minutes to allow aerosols to settle.
 - 10.14.2. Put on PPE.
 - 10.14.3. Apply disinfectant to all contaminated surfaces within the chamber, taking care to minimize splashing.
 - 10.14.4. The contact period should be appropriate for the disinfecting agent used in the process and then complete clean-up of the chamber.
 - 10.14.5. Remove buckets and rotors to nearest Biological Safety Cabinet; disinfect and clean as per manufacturer's instructions.

11. REFERENCES

- 11.1. Biosafety in Microbiological and Biomedical Laboratories, CDC-NIH, 6th edition, Appendix A: Available at:
https://www.cdc.gov/labs/pdf/SF__19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf.
- 11.2. Standard Operating Procedure Biosafety Level 2 (BSL-2), Available at:
<https://compliance.ouhsc.edu/Committees/Institutional-Biosafety/Biosafety-Standard-Operating-Procedures-SOPs-Forms>