



Safe Operating Procedure

(Revised 5/25)

WORKING IN A BIOLOGICAL SAFETY CABINET (BSC)

Introduction

This SOP applies to all laboratory spaces at UNL and affiliated campuses that use biological safety (biosafety) cabinets (BSC). A description of different types of biosafety cabinets and their characteristics can be found in the EHS SOP, ***Classification and Design of Biological Safety Cabinets (BSC)***. Information on decontamination of laboratory equipment can be found in the EHS SOP, ***Biological Decontamination of Laboratory Equipment***. This document details the accepted practices and procedures for working safely in a Biological Cabinet.

References

Biosafety in Microbiological and Biomedical Laboratories, current edition, Appendix A, Centers for Disease Control and National Institutes of Health

Proper use of a Biological Safety Cabinet

- Read and follow the operator's manual and all the manufacturer's recommendations.
- Biosafety cabinets should be thoroughly cleaned (work area and under the work area) at least every 3-6 months.
- Biosafety cabinets should not be placed in high traffic locations, under supply air vents, or near doors.
- Know which class of BSC you are using. Refer to the EHS SOP, ***Classification and Design of Biological Safety Cabinets (BSC)*** for more information about different classes of biosafety cabinets.
- A laboratory coat (preferably with elastic cuffs) and gloves must be worn when working in the BSC. Gloves should be pulled over the knitted wrists of the gown or lab coat. If two pairs of gloves are worn, put the first pair under and the second pair over the wrist/cuff of the gown or lab coat. Elasticized sleeves can also be worn to protect the investigator's wrists.
- Once work has started, remember to disinfect the gloves and provide proper contact time (if double gloving) OR remove the gloves prior to removing your hands from the cabinet.

- **Limit movement in and out of the BSC.** Repeated movement of arms into and out of the cabinet can disrupt airflow and affect cabinet performance. When it is necessary to move arms in and out of the cabinet, this should be done slowly and with movement perpendicular to the face of the cabinet.
- Work at least 4" to 6" behind the front grille/ air vent of the cabinet. Work as far back in the cabinet as is feasible.
 - Arrange materials and equipment so that they do not block grilles/vents. If possible, place equipment that may vibrate, rotate, disrupt airflow or generate heat in the rear area of the cabinet.
- Only materials and equipment required should be placed in the BSC.
 - 7 things should always be in the BSC when you are working with infectious materials: Biowaste bag (located in a secondary container); liquid catching container (and biowaste bag to contain it before removal from the BSC); absorbent liner(s); pathogen specific disinfectant; towels to wipe down materials/ surfaces; extra gloves; autoclave tape
- Contaminated liquid waste must never be poured directly into biohazard autoclave bags as autoclave bags are not durable and will leak.
- It is best practice to line a waste container with an autoclave bag to hold directly contaminated trash generated within the BSC.
 - We do NOT recommend taping the bag to the wall of the BSC unless a full BSC clean up under the vents/ grilles is occurring.
- All waste generated in the biosafety cabinet should be placed into a biohazard bag, discard tray or other suitable container prior to removal from the biosafety cabinet. Additionally, these containers should be surface decontaminated prior to removal from the biosafety cabinet. The trash cans on the floor beside the BSC are NOT for biological waste.
 - Add a small amount of water to biohazard bags prior to sealing them in the cabinet to generate steam when autoclaved. If EHS is picking up your waste, this step can be skipped.



Do not remove contaminated pipettes or tips from the cabinet to place in a biohazard bag or disinfectant bath outside the cabinet. This is unnecessary if a disinfectant tray is used inside the cabinet and contrary to the guidance provided above about limiting movement in and out of the cabinet.

- Use good microbiological techniques to minimize aerosolization.

- A sharps container must be used for sharps. Sharps are not permitted to be disposed of in pipet boats/ sterilization pans or autoclave bags. (Refer to EHS SOP, **Sharps Handling and Disposing**)
- Liquid catching containers/ pipette boats/sterilization pans should be used when serological pipets are used in the BSC.
 - Serological pipets and pipet tips should be placed in these durable containers (pipet boats) containing enough agent-specific disinfectant to cover the material placed in it.
 - Liquid catching containers/ pipette boats/sterilization pans are also good for catching drips, disinfecting and discarding pipette tips and disposable loops, and are good for placement of unused and discarded liquid material.



- Work from “clean” to “dirty” areas. Place trash/ infectious material toward the rear of the cabinet.
- **Restrict use of the cabinet to one (1) person at a time.** A risk assessment and consultation with the Biosafety Officer is necessary to allow two people to work simultaneously in the same BSC.
 - When approved, two persons working in a BSC is limited to 6 foot BSCs with no greater than an 8” sash height.
- Use disposable loops and spreaders to avoid the need for heat sterilization methods.
 - If you need a heat source, use a touch-activated gas burner. Touch-activated gas burners are activated only when a touch sensitive switch is activated.
- **DO NOT** use Bunsen burners in the BSC.
 - Natural gas, a flame, and alcohol-based disinfectants do not work well together!
 - Heat sources in the biosafety cabinet are disruptive to the air flow in the cabinet and create turbulence! This disruption can lead to contamination of samples and potential release of contaminants from the cabinet.
 - The flame and heat generated damages the HEPA filter above the work surface.
- Do not allow people to walk behind BSCs when work is in progress
- Do not block the front or back air vents/ grilles.

- Avoid moving your hands in and out of the cabinet when work is in progress.
- Do not place serological pipets directly into autoclave bags- they will puncture the bag.

Working in the BSC

1. Setting up BSC for work with infectious substances:

- a) If the cabinet is turned off before you begin work, turn the blower/ motor on and allow it to warm up for 3-5 minutes while you are cleaning and setting up your supplies. Ensure the cabinet is operating correctly and no alarms are activated before beginning work.
- b) Place the sash at the correct level (arrow marker to the left of the sash or look in instruction manual for optimal sash height).
- c) Turn on the BSC light and disinfect all BSC surfaces (side walls, back, inner sash and work area) with appropriate disinfectant.
 - i. Use a Swiffer™ sweeper or equivalent to wipe down the hard-to-reach areas like the back and side corners of the BSC.
- d) Lay down absorbent liner(s) on the BSC work area, careful not to cover or block the front or back air vents/ grilles.
- e) Place a solid waste container containing a biohazard autoclave bag on the “dirty” side of the cabinet.
 - i. Example, if you are right-handed, your dirty side would be on your left for in vitro work.
- f) Prepare a pipet boat or other disinfectant type liquid catching container for organism and liquid material containment.
 - i. Place pathogen specific disinfectant into the liquid catching container/empty pipet tip box/ pipet boat/sterilization pan if the disinfectant is safe to be autoclaved.
 1. Add enough disinfectant to cover whatever is being added to it.
 2. Pipet boats are needed to hold used pipets but are also good for catching drips and containing other materials such as pipette tips and disposable loops.
- g) Place pathogen specific disinfectant, paper towels, and extra gloves on the “clean” side of your work area.
 - i. Example, if you are **right**-handed, your clean side would be on your right side for in vitro work.
- h) Everything needed to complete the particular procedure should be placed inside the cabinet prior to beginning work.



- i. Arrange implements in a logical manner to provide a “clean” to “dirty” flow of materials.
 - 1. Example, if you are **left**-handed, your clean side would be on your left side and your dirty side would be on your right-hand side for in vitro work.
 - ii. Be mindful of the placement of materials or equipment inside the cabinet. Excess items blocking the vents at the back or front of the cabinet will cause disruption of airflow, resulting in turbulence, possible cross-contamination, and/or breach of containment.
- i) Adjust the seat/ stool height so that your arms are not blocking or resting on the front grille/ air vent, and your face is above the front opening.
 - j) Place sharps container in BSC, if applicable.
 - k) Work must be conducted at least 4 inches back from the front grille/ air vent.
 - i. The middle towards the back of the work surface is the ideal area to be used for safety of you, your lab and your sample.
 - l) Limit arm movements and move slowly so as not to disrupt airflow unnecessarily.
 - m) Place supplies that may be needed on a cart or in drawers right next to the BSC so you do not have to get up from the cabinet and disturb the air currents.
 - n) Let the cabinet warm up for at least 5 min. before starting any open culture work.
 - i. This time can include the set up and cleaning time.
 - ii. Before actually starting work, wait for 30 seconds to let the cabinet air balance.

2. Trash in BSC:

- a. Line a small bucket or container with a biohazard autoclave bag (disinfectant/ water applied inside the bag) to hold solid waste generated within the BSC.
 - i. **DO NOT block airflow from the front or back grilles/ air vents!**
- b. Liquid waste should be placed in a durable liquid catching container containing disinfectant.
 - i. If the volume is not too large, a centrifuge tube, or empty media bottle with disinfectant in it can be used.
- c. Sharps containers must be placed inside the BSC and sealed in an autoclave bag or Ziploc style bag before exiting the BSC **for organisms transmitted via aerosol.**

3. Working in the BSC:

- a. To avoid the creation of aerosols.
 - i. **Try not to** blow out the last drops of liquid when pipetting. If not possible, dispense SLOWLY!
 - ii. Dispense liquid against the inside of a container to prevent splashing and aerosolization.
 - iii. Place pipette tip under liquid until liquid is dispensed.
 - b. Loosen lids enough to be able to get the lid off when the time comes to use the container.
4. **When work in BSC is completed:**
- a. All equipment which has been in the BSC with the biological material needs to be enclosed or **surface decontaminated** with a paper towel sprayed with appropriate disinfectant before it is moved over to the “clean side” of the BSC for proper contact time. Once contact time is achieved, it can be slowly removed from the cabinet and placed on a cart or lab space near the BSC.
 - b. For liquid catching container/ pipette boat:
 - i. Place the lid onto the container and secure it with autoclave tape, if applicable (Pipette boat lid)
 - ii. Properly bag the liquid catching container/ pipette boat in a “biohazard” labeled autoclave bag and seal the bag with autoclave tape.
 - c. Wipe down container(s) with appropriate disinfectant.
 - d. Decontaminate the BSC by disinfecting all surfaces with pathogen specific disinfectant.
 - e. All biological waste, once properly bagged in biohazard labeled autoclave bags, must be **transported in a secondary container** (autoclave bin, transport carts, etc) to the autoclave or approved staging area.
 - f. All sharps containers that will be used again and kept in the lab must be contained in an airtight sealed bag, if it contains biological material that is infectious via aerosolization (e.g. autoclave bag or Ziploc style bag sealed airtight).

Chemical and Radioisotope Use in BSCs

Class I, Class IIA and Class III BSCs are not designed for use with volatile chemicals or radionuclides. HEPA filters will not “trap” vapors, gases or fumes.

HEPA filters, gaskets, and their housing assemblies can also be damaged by some chemicals thereby compromising workers and environmental protection from the biological products worked with in the BSC.



BSCs are not designed with sealed electrical components (i.e., switches, lights, etc.). If sufficient concentrations of flammable vapors are present in the cabinet (i.e., at or above the Lower Explosive Limit, LEL), a fire or explosion may result.

Relatively small amounts of non-volatile chemicals and radionuclides can be used in all BSCs, but amounts should be restricted to minute quantities.

Small quantities of volatile chemicals can be used in a Class II, Type B2 biological safety cabinet..

UV Lights in Biological Safety Cabinets¹

UV lamps are **not recommended, nor required** in biological safety cabinets according to the Centers for Disease Control (CDC), the National Institute of Health (NIH) and the BSC manufacturers. Contact the Biosafety office for a risk benefit analysis.

Biosafety Cabinet Certification

Regardless of classification, biosafety cabinets must be certified by a qualified technician according to the current NSF/ANSI Standard 49. This is to ensure proper airflow and HEPA filtration within the cabinet. Certification must occur:

After initial purchase and installation.

After relocation.

Annually, after installation.

Do not use a cabinet that has not been certified, missing a certification sticker (or the sticker is illegible) or is past due for annual recertification.
