ENVIRONMENTAL HEALTH AND SAFETY
University of Nebraska-Lincoln

Safe Operating Procedure

(Revised 5/24)

WORKING IN A BIOSAFETY CABINET

Introduction

This SOP applies to all laboratory spaces at UNL and affiliated campuses that use biosafety cabinets (BSC). A description of different types of biosafety cabinets and their characteristics can be found in the EHS SOP, **Biosafety Cabinets**. This document details the accepted practices and procedures for working safely in a Biosafety Cabinet.

The information provided in this SOP supplements the UNL Bloodborne Pathogen Exposure Control Plan and UNL Biosafety Guidelines. Please refer to the full program documents for more information.

References

Biosafety in Microbiological and Biomedical Laboratories

Proper use of a Biological Safety Cabinet

Prior to Work Starting

- Read the operator's manual and follow all the manufacturer's recommendations. Know which class of BSC you are using. Refer to the EHS SOP, **Biosafety Cabinets** for more information about different classes of biosafety cabinets.
- Seat/stool height should be adjusted so that the investigator's face is above the front opening.
- If the cabinet is off before you begin work, turn it on and allow it to run for 3-5 minutes while you are preparing your supplies. Ensure the cabinet is operating correctly and no alarms are activated before beginning work.
- **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. Two persons working in a BSC is also limited to 6' or larger BSCs with no greater than an 8" sash height. This guidance is based on a recent unpublished study conducted by biosafety cabinet manufacturer Baker[1].

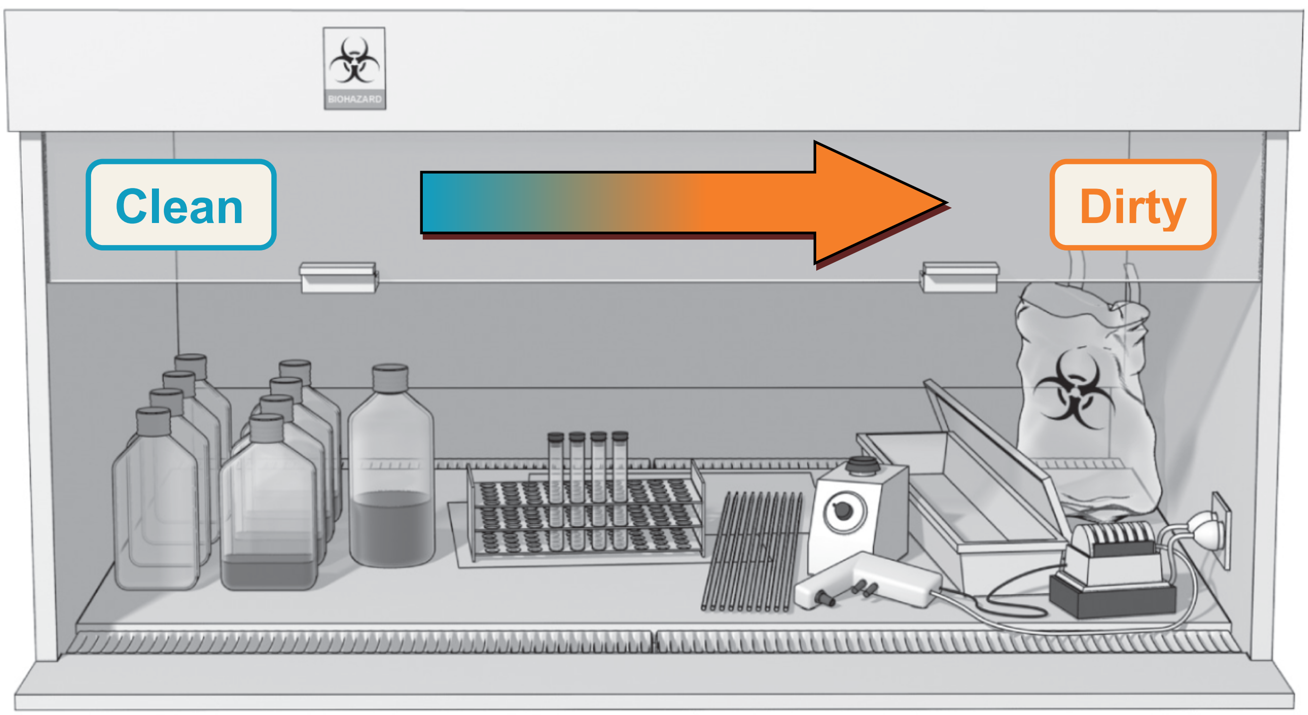
- Disinfect all interior surfaces (back and side walls, work surface and back of sash) of the BSC with an appropriate disinfectant.
- Be mindful of the placement of materials or equipment inside the cabinet. Excess items blocking the vents at the back or front of the cabinet may cause disruption of airflow, resulting in turbulence, possible cross-contamination, and/or breach of containment. Extra supplies (e.g., extra gloves, culture plates or flasks, culture media) should be stored outside the cabinet.
- Verify proper pressure differential (generally magnahelic reading) to detect potential loading, tear, or other problem with the HEPA filters.

Working in the BSC

- Wipe the surfaces of items placed in the cabinet with 70% ethanol or 70% isopropanol or another appropriate disinfectant. This will reduce the introduction of contaminants into the cabinet environment.
- Arrange materials and equipment so that they are in logical order, they do not block grills, and they are at least 4 inches from the front grille of the working surface. Place items to be used first closer to you and dirty materials on the opposite side of the cabinet from the clean materials. Potentially infectious organisms or cultures should be the last item added to the BSC and the first item to be decontaminated and removed. If possible, place equipment that may vibrate, rotate, or generate heat in the rear area of the cabinet. Applied research confirming this practice was recently conducted by Baker.[2]
    - Be sure to include a tray of liquid disinfectant for pipettes and tips when working with infectious materials.
    - A small biohazard bag should also be located inside the cabinet to dispose of any solid waste generated during the procedures.
- ⚠ **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. It is advisable to use a checklist to ensure that all necessary items for a specific activity have been placed in the cabinet prior to starting a procedure.
    - 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. Do not block the front grille by resting arms on them or by putting notes or any other materials on them.
    - Once work has started, remember to disinfect gloved hands prior to removing them from the cabinet.
- Manipulation of materials should be delayed for one minute after putting arms into the hood to allow the hood environment to stabilize.

- Work at least 4" to 6" behind the front grille of the cabinet. Work as far back in the cabinet as is feasible since exhaust air is primarily drawn from the back of the cabinet.
- Personal protective clothing such as a laboratory coat (preferably with elastic cuffs) and gloves must be worn when working at the hood. A solid front and back closing gown provides the best protection. 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 of the gown or lab coat. Elasticized sleeves can also be worn to protect the investigator's wrists.
- Use good pipetting technique to avoid production of aerosols. Discard pipettes in a tray of disinfectant inside the cabinet, and other small materials (pipette tips, closed tubes, etc.) in a biohazard bag inside the cabinet.

Figure 1 A typical lay out for working "clean to dirty" within a Class II BSC.[3]



Clean cultures (left) can be inoculated (center); contaminated pipettes can be discarded in the shallow pan and other contaminated materials can be placed in the biohazard bag (right). This arrangement is reversed for left-handed people.

- Work from "clean" to "dirty" areas. Place contaminated materials toward the rear of the cabinet. (Figure 1)
- Clean up spills as soon as they occur. If a large spill occurs, stop work, secure all agents and remove all materials from the cabinet following the procedures outlined below in the

"Finishing Work in a BSC." Then follow the decontamination procedures for a BSC outlined in the EHS SOP, **Biological Decontamination of Laboratory Equipment**.

- 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.
  - 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.

Finishing Work in the BSC

1. Disinfect non-disposable equipment and supplies.
2. Disinfect gloves and sleeves (if worn) and remove hands and arms from the cabinet.
   a. If two pairs of gloves are being worn, simply remove the outer pair of gloves without contaminating the inner pair and place them in the biohazard bag. Then remove your hands from the cabinet and don a fresh pair of gloves.
3. After the appropriate contact time for the disinfectant has elapsed, retrieve cleaned equipment from the cabinet.
4. Once all materials have been removed from the cabinet, disinfect the cabinet surfaces with a disinfectant that is appropriate for the agent in use. Refer to the IBC protocol for a list of approved disinfectants that are specific to the protocol. Be sure to allow sufficient contact time for the disinfectant, as indicated on the product label.
5. Leave the fan blower on in the cabinet for a short period of time (5 to 10 minutes) after finishing work to allow the system to purge. Some safety cabinets should be left on at all times. Check with your supervisor for proper operating procedures.

Maintenance and Relocation of BSCs

- Never attempt to remove or change the HEPA filters or perform any modifications or maintenance. All such work must be performed by a qualified BSC technician.
- Biosafety cabinets must be decontaminated before being moved or relocated. Please refer to the EHS SOP***, Biological Decontamination of Laboratory Equipment,*** for advice on the necessary method of decontamination. If pathogens or human cell lines were used in

the cabinet, gas decontamination is required in addition to surface decontamination of the cabinet.

- Refer to the EHS SOP, **Biosafety Cabinets** for guidance on proper placement of BSCs in a room for optimal operation.

Use of Heat Sources or Open Flames in Biological Safety Cabinets

A study conducted by biosafety cabinet manufacturer Baker showed that heat sources in the biosafety cabinet are disruptive to the laminar flow of air in the cabinet.[4] This disruption can lead to contamination of samples and potential release of contaminants from the cabinet.

The hazards associated with flames and other heat sources in the biosafety cabinet are three-fold.

1. There is a concern of buildup of natural gas in the cabinet if the supply of gas is left on without a flame burning. If this occurs, any spark could ignite an explosion, which would severely damage the cabinet and seriously injure any lab workers nearby.
2. The heat from the flame or the electric incinerator will disrupt the laminar flow of air in the cabinet workspace.
3. The flame and heat generated can damage the supply HEPA filter above the work surface after prolonged exposure. The table below indicates which types of flame/heat sources are allowed in BSCs at UNL.

Flame or Heat Source	Allowed
Bunsen Burner	**No** 👎
High-temp Bunsen Burner (aka Fisher Burner)	**No** 👎
Spirit (alcohol) lamp	**No** 👎
Electric incinerator (Bacti-cinerator®)	**No*** ⚠
Touch-activated gas burners (Touch-o-matic®, FireStar™)	**Yes** 👍

**Acceptable if operated only when needed within the cabinet. And turned off when not in use.*

Best Practice Recommendations:

- Use disposable loops and spreaders to avoid the need for heat sterilization methods.
- Touch-activated gas burners are activated only when a touch sensitive switch is activated. These devices prevent an unlit burner from filling the cabinet with gas, which is possible with a regular Bunsen burner. They also limit the time the flame is on in the cabinet, which minimizes the impact on airflow and potential for damage to the HEPA filter.

Chemical and Radioisotope Use in BSCs

In general, BSCs are not designed for extensive use with volatile chemicals or radionuclides. HEPA filters will not "trap" vapors, thereby exposing laboratory workers when the vapors are discharged back into the laboratory and picked up by the ventilation system.

- HEPA filters, gaskets, and their housing assemblies can also be damaged by some chemicals thereby compromising workers and environmental protection.
- 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.
- The low face velocity of Class I and Class II, Type A1 BSCs is insufficient to contain chemical vapors.
- Relatively small amounts of non-volatile chemicals and radionuclides can be used in all BSCs, but amounts should be restricted to minute quantities when using a Class II, Type A1 BSC (due to the low face velocity and design of discharge air).
- Small quantities of volatile chemicals can be used in a Class II, Type B2 and Class III BSCs. The amount in process should never be in a quantity that can generate vapor concentrations at or near the LEL.

Use of UV Lights in Biological Safety Cabinets[5]

The Centers for Disease Control (CDC) and the National Institute of Health (NIH) agree that UV lamps are **not recommended, nor required** in biological safety cabinets. The activity of UV lights for sterilization/decontamination purposes is limited by a number of factors, including[2,4]:

- ***Penetration*** - In a dynamic air stream (e.g., biological safety cabinet), UV light is not penetrating. Microorganisms beneath dust particles or beneath the work surface are not affected by UV irradiation. UV irradiation can cause erythema that may damage both the skin and eyes of laboratory employees. The surface and outer tissue layers of eyes and skin are primarily involved because UV does not penetrate deeply into tissue. These effects are generally not permanent but can be quite painful.

- ***Relative Humidity*** - Humidity adversely affects the effectiveness of UV. Above 70% relative humidity, the germicidal effects drop off precipitously.
- ***Temperature and Air Movement*** - Optimum temperature for output is 77°- 80°F. Temperatures below this optimum temperature result in reduced output of the germicidal wavelength. Moving air tends to cool the lamp below its optimum operating temperature and therefore results in reduced output.
- ***Cleanliness*** - Dust and dirt can block the germicidal effectiveness of ultraviolet lights.
- ***Age*** – The amount of germicidal wavelength light emitted from these bulbs decreases with age and bulb ratings (hours of use) may vary by manufacturer.

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, is missing a certification sticker (or the sticker is illegible) or is past due for annual recertification.

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[1] https://absaconference.org/wp-content/uploads/2020/02/ABSA2019_Session_XVIII_1415_1435_Held_K.pdf https://bakerco.com/communication/bsc-mythbusters/

[2] Held, K.F. Boudreau, J. (2019). *Can overcrowding a BSC work area lead to a loss of containment?* [White Paper]. Baker, Inc. https://bakerco.com/communication/bsc-mythbusters/

[3] Centers for Disease Control and Prevention, N. I. Biosafety in Microbiological and Biomedical Laboratories (Current edition). Washington, DC: U.S. Government Printing Office.

[4] Held, K. F., Thibeault, R., & Boudreau, J. (2019). Heat Sources in a Biosafety Cabinet Compromise Experimental and User Protection. *Applied Biosafety*, *24*(2), 90–95. https://doi.org/10.1177/1535676019831173

[5] Burgener, J. (2006). Position Paper on the Use of Ultraviolet Lights in Biological Safety Cabinets. Applied Biosafety , 11 (4), 228-230.