

Working Safely in a Biological Safety Cabinet (BSC) for BSL-2 SOP

California State University Fullerton • Environmental Health & Safety
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Originator: Environmental Health and Safety Office

1.0 Purpose

The purpose of this document is to enhance safety at CSUF by ensuring that everyone working in a biosafety cabinet (BSC) is aware of the measures that are in place to protect them and avoid potential exposure to biohazardous materials. The work practices described in this document will also help maintain sterile conditions when working with all types of cell culture and other non-hazardous materials that need to be kept sterile.

2.0 Scope

This procedure describes the appropriate way to handle biohazardous and potentially biohazardous materials in a BSC at a BSL-2. The BSC should be used with human-sourced materials, infectious agents, cell cultures, and for any other materials where aerosol containment and maintaining sterility is desired.

3.0 Definitions

BSC – A **biological safety cabinet** is the primary means of containment developed for working safely with infectious microorganisms; it is an enclosed, ventilated laboratory workspace under negative pressure. Depending on the class of the BSC, it protects the user and co-workers, and may protect the material being handled inside the cabinet.

Class II BSC – The Class II BSC are partial barrier systems that rely on the directional movement of air (laminar flow) through a HEPA filter to provide containment of potentially infectious aerosols generated within the cabinet and also to provide product protection. All cabinets in this class are designed for work involving microorganisms and provide the microbe-free work environment necessary for cell culture propagation. A class II BSC provides protection to the user and co-workers, the environment, and the material being manipulated.

Class II, Type A1, A2 – This type of BSC recirculates approximately 70% of the HEPA filtered air through a supply filter and exhausts approximately 30% of the air through an exhaust filter to the room or through a direct connection to the facility exhaust system. This type of cabinet does not provide protection from vapors and gases.

Class II, Type B1 – This type of BSC recirculates approximately 30% of the HEPA filtered air through a supply filter and exhausts 70% of the air through an exhaust filter hard ducted to the outside. Minute amounts of volatile toxic chemicals may be handled in this cabinet; exhaust from the rear of the cabinet are exhausted outside, so work with these materials should be restricted to the back of the cabinet.

Class II, Type B2 – This type of BSC is a total exhaust cabinet whereby no HEPA filtered air is recirculated into the work area. 100% of the air is exhausted to a hard-ducted exhaust system to the outside. This type of cabinet provides protection from vapors and gases.

HEPA filter – **high-efficiency particulate air** or HEPA is a type of air filter found in BSC that remove 99.97% of particles with a size of 0.3 µm or larger from the air passing through it.

Laminar airflow – a system of circulating filtered air in parallel-flowing planes used in a biological safety cabinet which reduces the risk of airborne contamination and exposure to chemical pollutants. (See Appendix A of this document for diagram of air flow in a BSC)

Magnehelic gauge – a differential pressure gauge showing relative pressure differences between two points which serves as a visual confirmation that the biosafety cabinet is operating properly. Routine monitoring of the magnehelic gauge values could help indicate a blockage or tear in the HEPA filters which would interfere with the correct functioning of the BSC. (See Appendix A of this document for image)

Swiffer® - A type of dry dusting cleaner commercially available in many formats to assist with cleaning the BSC cabinet. (See Appendix of this document for image)

4.0 Procedure

4.1 General Principles

- A biological safety cabinet is designed to provide protection to the operator, the environment and the product. Protection to the operator is provided via the inward flow of air through the front grill. This air barrier is very sensitive and can be compromised by movement in or near the cabinet, heat, equipment that may generate air turbulence, blockage of vents or grills, and overcrowding of the interior of the unit. The BSC provides protection to the product (work materials) by providing HEPA filtered air onto the work surface. Environmental protection is provided by HEPA filtration of the exhaust air.
- Only the materials and equipment required for the immediate work should be placed in the BSC. Extra supplies (e.g., additional gloves, culture plates or flasks, culture media) should be stored outside the cabinet. Cluttered conditions inside the cabinet while working contribute to disruption of the laminar air flow. When not in use, the BSC should be as empty as possible.
- A BSC should never be used when an alarm is sounding, a warning light is flashing, or you detect a problem with the airflow. Check the magnehelic gauge, if present, before use – if it is on “0”, do not use the cabinet, as it may have a hole in the HEPA filter.

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- Prior to using the BSC, check for a current certification sticker. If there is no sticker or the sticker is out of date, contact the EHS Office to coordinate/schedule the certification. The BSC needs to be certified when newly installed, at least annually thereafter or whenever moved or repaired. In addition, the BSC will be decontaminated professionally prior to moving or decommissioning.
- The BSC is not a chemical fume hood; work with volatile or toxic chemicals only in a Class II Type B1 or B2 BSC (the amounts of volatile and toxic chemicals that can be handled will depend on the BSC model). With Class II A BSCs, there is increased risk of exposure to potentially hazardous chemical vapors because air is recirculated within the cabinet, and vapors may concentrate. Also, volatiles will not be stopped by the HEPA filter, so if a cabinet exhausts into the room, personnel can be exposed to volatiles. If tasks require the use of any volatile or toxic chemicals, use a fume hood or contact the Biosafety Officer (BSO) to discuss alternatives.
- Do not use the top of the BSC for storage. The HEPA filter could be damaged and the airflow disrupted.

4.2 Prior to Working in the BSC

- Turn off the ultraviolet light if the unit has one. NOTE: the use of ultraviolet lights is not recommended. Reflected UV can damage retinas. The UV light does not replace a thorough cleaning before/after every use of the BSC. If you want to use the UV light, it must be tested/replaced ~ every 3 months and should be dusted regularly. Remember that UV light does not penetrate items and will only affect the surfaces nearby. (See Reference #2 of this document.)
- Turn on the BSC if it is off by pressing the blower on/off switch. NOTE: Many units are designed to continuously run 24 hours a day.
- Put on gloves and a lab coat at a minimum. Long hair should be tied back, and cell phones should not be handled while working in the BSC.
- Wipe down the entire BSC (walls, work surface, glass) with a disinfectant – a disinfectant is recommended if someone else used the unit before you, and you are not sure they did a thorough cleaning job. Appropriate disinfectants include 0.1% sodium hypochlorite bleach (freshly made daily), Cavicide, or other disinfectant listed for surface disinfection in *Selection and Use of Disinfectants SOP*.
 - **NOTE:** A dry Swiffer® can be used very effectively for the cleaning step – spray the disinfectant on the dry pads. Use a new pad before and after working.

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- Load the cabinet with only the articles needed for the first part of the procedure (e.g., until you would get up to place something in the incubator, refrigerator, centrifuge). This is so that the cabinet is not overloaded. It is handy to have a small biohazard bag inside the cabinet for paper towels, pipet wrappers, glove disposal, etc.
- Position materials and equipment to avoid blocking any air vents or grills. Do not ever place anything on the front grill. Blocking the grills at the opening of the BSC may allow contaminated air from inside the cabinet to enter the user's breathing zone, and also allow contaminated room air to enter the cabinet without filtration.
- Arrange items inside the cabinet so that you will not cross hands. For example, if you are right-handed, the pipet aid should be on the right side of the cabinet, serological pipets on the left, pipet tip boxes on the right, discard beaker/pan in the middle or right side of the cabinet. Avoid crossing hands, withdrawing hands, chair movement as much as possible. Place the disinfectant of choice in a beaker or flat pan inside the cabinet. Place paper towels and a spray bottle of the disinfectant inside the cabinet before starting work. This will be used for spills, spraying gloves and removing materials.
- Confirm that the BSC alarm is operational and the sash is at its proper position. Never disable the BSC alarm (which indicates improper airflow), since improper airflow affects performance and could endanger the researcher or the experiment. Position the chair so that the operator is looking down (through the sash glass) onto the work surface. The chair must not be so low that the user is looking under the sash at the work surface.
- Do not open anything inside the cabinet or perform any work for 5-10 minutes, as this time is needed to purge any contaminants inside the BSC before work begins.

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4.3 Working in the BSC

- Use good microbiological techniques appropriate for working in the BSC to avoid aerosol generation, splatter and cross contamination. Good microbiological techniques include:
 - Arrange materials inside the BSC to segregate contaminated and clean items. Avoid movement of contaminated items over clean items. The general workflow should be from clean to dirty (contaminated):

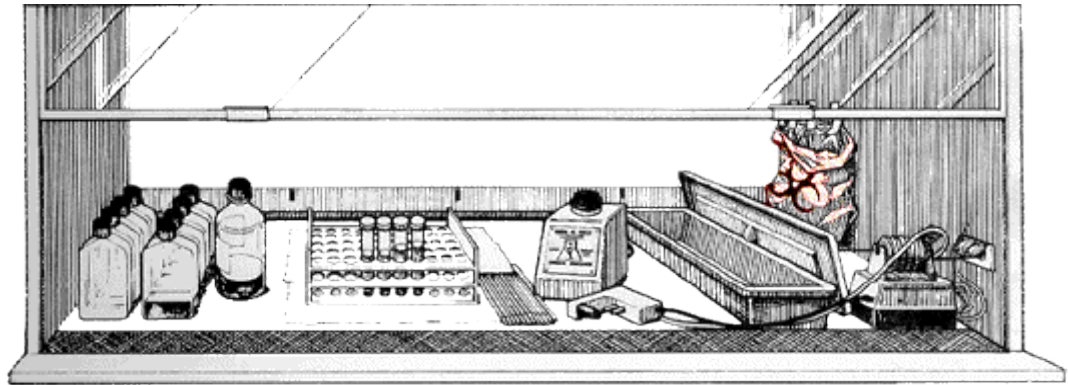


Figure 1.1: Working “Clean to Dirty” within a Class II BSC. Clean cultures (*left*) can be inoculated (*center*); contaminated pipettes can be discarded in the shallow pan and other contaminated materials can be placed in the biohazard bag (*right*). This arrangement is reversed for left-handed persons. You may find it easier ergonomically to have the discard pan/beaker in the middle or just right of middle. The small biohazard bag would be better on the left side.

- If appropriate, work on absorbent toweling to reduce splatter and aerosol formation. When discarding pipets and tips, draw the disinfectant up inside the pipet, so that the inside is decontaminated.
- Work at least 6 inches in from the front of the BSC. Manipulation of materials should be delayed for approximately one minute after placing the hands/arms inside the cabinet if possible – this will allow the BSC to stabilize and to “air sweep” the hands and arms to remove surface microbial contaminants. Keep arms as far inside the BSC as is comfortable. Work as far towards the back of the BSC as is comfortable – it is safer for the contaminated air to go out via the rear vent than the front grill.
- Keep arms inside the BSC while work is ongoing. Keep movements slow and at a minimum in the cabinet to avoid generating turbulence. Do not reach outside the cabinet for pipets and other supplies, or to discard materials. Every time your hands leave and re-enter the cabinet you risk pulling out infectious aerosols and/or

introducing dirty unfiltered air into the cabinet.

- Do not use Bunsen burners in the unit, as the heat disrupts airflow and may damage the filtration system. Instead, use a microburner, electric incinerator, or disposable plastic inoculating needles/loops.
- Do not mouth pipet, eat, drink, smoke, apply cosmetics, or handle contact lenses while working in the BSC.
- To reduce the chances for cross contamination, hold open tubes or bottles at a slight angle. Items should be recapped or covered as soon as possible. If possible, avoid putting down caps and lids, since this increases the chance that they will become contaminated.
- When a vacuum is used inside the cabinet (either the house vacuum system or a vacuum pump), the aspiration system must be connected to a liquid disinfectant trap and a filter capable of capturing the organisms being handled and suitable for use in vacuum lines. See *Working at Biosafety Level 2 (BSL-2) SOP* for information on setting up a vacuum system.
- Keep room activity in the vicinity of the BSC to a minimum.
- Spills inside the BSC must be handled per the spill procedure in Section 4.6.
- Aerosol generating equipment (vortex mixers, blenders, cell homogenizers, bead beaters, small centrifuges, tissue grinders, etc.) should all be used inside the BSC, particularly when human-sourced materials or infectious agents are involved. BSCs may not provide sufficient containment for large equipment that generates turbulence, e.g., a large clinical centrifuge. If you plan to use a larger piece of equipment inside the BSC, pre-test the ability to maintain containment by running the equipment (without samples) inside the cabinet and testing the airflow with dry ice and water, a smoke stick, etc., to confirm that any aerosols will be contained.

4.4 At the Completion of Work in the BSC

- Consider all materials inside the BSC to be contaminated. Remove live cultures or plates by wiping down with disinfectant-soaked paper towels. Remember to spray gloves or remove gloves before removing hands from the BSC. Discard all tubes and other plastic contaminated items into the disinfectant container (make sure they are submerged and all surfaces are wet). If you are using a beaker with disinfectant, spray the tops of the pipets with disinfectant. Disinfect all equipment (spray or wipe) before removal. Close the small biohazard bag with tape and spray outside with disinfectant -- remove and place in biohazard waste container. Wipe down the outside of the disinfectant beaker/pan and remove -- allow at least 15 minutes of contact time for the items in this container. Drain the disinfectant into the sink (a colander works well) and place decontaminated

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disposables into the biohazard waste bin or pipets into a lined cardboard box/broken glass box to avoid puncturing waste bags.

- When the inside of the BSC is empty, wipe down all interior surfaces of the BSC with the disinfectant (not alcohol). Use the *Swiffer*[®] if you have one. If you use a bleach solution, you should follow the bleach wipe-down with alcohol or sterile water to remove the bleach residue and avoid pitting of the stainless steel.
- Check the trough under the front grill for spilled material. Clean and disinfect the trough and grill whenever spillage is noted.
- Let the BSC run for at least 5 minutes before shutting it off (to purge the inside).
- The work surface should be removed periodically (if the BSC has a removable surface) and all exposed surfaces disinfected.
- Follow BSC manufacturer directions for whether or not to close the window sash with the motor running.

4.5 Emergency Procedures

- If an alarm sounds or the unit failure alarm is activated while you are working in a BSC, close or bag up biohazardous materials. Remove your hands very slowly from the BSC. Check to see if the BSC has been overloaded or air grilles blocked causing a problem with airflow. If system still alarms notify the PI and Facilities Operations Service Center. Post a sign on the BSC indicating that it is not functional and silence the alarm if you have access to it.

4.6 Spill Cleanup in the BSC

- Change PPE – consider your gloves and sleeves contaminated. It is not necessary to evacuate the lab unless the infectious material went outside of the BSC.
- For a small spill, wipe the spill up with a paper towel, followed by an aqueous detergent, then wipe with a disinfectant.
- For a larger spill, cover the spill with absorbent material (paper towels), flood area with appropriate disinfectant (10% bleach or other tuberculocidal disinfectant – NOT alcohol). Allow >15 min for disinfectant to be effective. Discard paper towels into the biohazard waste bin, clean the area with an aqueous detergent, and then with the disinfectant. Discard all cleanup materials in the biohazard waste bin.
- Decontaminate all materials/items inside the BSC.

- After disinfecting supplies and equipment, remove, and clean all surfaces of the BSC thoroughly.
 - For Class II Type A cabinets, you should remove the work surface and clean underneath, as well as the bottom of the work tray, particularly if the spill involved infectious material. If you only spilled media, you would still want to consider cleaning under the work surface, to prevent mold growth.
- Place all waste in red biohazard waste bag.
- Change PPE.
- Resume work.

5.0 BSC Maintenance, Move, Repair and Decommissioning Procedures

- 5.1 The BSC must be certified by an NSF/ANSI Standard 49 qualified technician when newly installed, at least annually thereafter or whenever moved or repaired. In addition, the BSC will be decontaminated professionally before decommissioning.
- 5.2 The decontamination methods include fumigation with vaporized hydrogen peroxide, chlorine dioxide gas or formaldehyde gas. BSC decontamination may only be performed by a certified contractor.
- 5.3 Prior to certification, repair and/or professional decontamination, the laboratory users are responsible for wiping all internal surfaces of the BSC with a disinfectant, as described in section 4.4. After this wipe down is completed, complete and post the Equipment Decontamination Verification Sticker (see Appendix B) on the BSC. Do not use the BSC after it is wiped down.
- 5.4 If the BSC is to be relocated or scrapped, prepare the unit as described in 5.2, post the Equipment Decontamination Verification label on it, contact one of the BSC Certifiers (EHS) to arrange for the professional decontamination and submit a work order to Facilities Operations Service Center for the move. Once the BSC is decontaminated, verify that it has a decontamination tag affixed from the vendor that performed the decontamination, and ensure that it is not put back into lab use while waiting for Physical Plant pick-up. Notify EHS that the unit is to be scrapped so documentation can be updated accordingly.

6.0 References

- (1) Biosafety in Microbiological and Biomedical Laboratories, CDC-NIH, 5th edition, Appendix A:
https://www.cdc.gov/labs/BMBL.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fbiosafety%2Fpublications%2Fbmb15%2Findex.htm
- (2) *Position Paper on the Use of Ultraviolet Lights in Biological Safety Cabinets*, *Applied Biosafety*, 11(4) pp. 228-230 © ABSA 2006.
<https://journals.sagepub.com/doi/abs/10.1177/153567600601100413>
- (3) Types of Class II Biosafety Cabinets, Baker Company.
<https://bakerc.com/communication/technology-overview/introduction-to-biological-safety-cabinets/>
- (4) *Selection and Use of Disinfectants SOP*
- (5) *Working at Biosafety Level 2 (BSL-2) SOP*

APPENDIX A

Magnehelic gauge



Swiffer®, The Proctor & Gamble Company, Cincinnati, OH



Baker Company, diagram of Class IIA2 BSC airflow:

Balanced Airflow and Exhaust Ensure Uniformity


The SterilGARD® e3 cabinet features a unique airflow design that delivers unidirectional downflow air over the work area for maximum containment and protection.

- Filtered air descends from top to bottom of the work area in a unidirectional flow. Near the back of the work surface, the air current divides – a portion of the downflow air is pulled through the back wall grille and the remainder is pulled through the front grille.
- Simultaneously, room air is pulled through the front opening and into the front grille. It does not enter the work area.
- All air combines under the work surface and is pulled under negative pressure through the back and side double walls of the cabinet into the blower/motor, which blows it into the positive-pressure preflow plenum.
- From the positive-pressure plenum, approximately 30% of the air exits the system through the exhaust filter. The remaining 70% passes through the supply filter and re-enters the work area as particulate-free air.

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APPENDIX B



CALIFORNIA STATE UNIVERSITY, FULLERTON

Environmental Health & Safety

Equipment Decontamination Form

Building: _____ Room Number(s): _____ Property Tag #: _____
 Principal Investigator: _____ Dept: _____
 Equipment Name: _____ Office/Lab Ext: _____
 Model: _____ Serial #: _____

I certify that the lab equipment mentioned above has been cleaned and decontaminated of all asbestos / chemical / biological / radioactive contaminants.
 (Circle all that apply)

 Principal Investigator's Signature (if available) _____
 Date

 EHS Staff (Signature) _____ _____
 EHS Staff (Printed Name) _____ Date

Additional Notes:

W:\EHS\General\6. Environment\Hazardous Materials Management\Equipment Decontamination Form

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