

The biological safety cabinet (BSC) is the primary means of containment to protect the

- User from exposure to biohazardous materials;
- Environment from releases of biohazardous materials; and
- Product from contamination.

Along with good standard microbiological techniques and safe work practices, BSCs are extremely effective and the single most important containment device in protecting laboratory workers and the immediate lab environment from exposure to pathogens, especially infectious aerosols. There are different classifications of BSCs (*see table below*), but all use directional inward airflow, and high efficiency particulate air (HEPA) filters to treat exhaust air. However, a risk assessment must be completed to determine the most suitable BSC for project needs.

### BSC Placement

The integrity of the directional air inflow through the front opening into a BSC is fragile and can be easily disrupted by air currents. To prevent air inflow disturbances, BSCs should be positioned in a remote location away from potentially disturbing air currents.

- Avoid placement in high traffic areas.
- Avoid potentially disruptive equipment such as air conditioners, chemical fume hoods, and ventilation systems such as air supply and exhaust units.
- Away from doors and windows that can be opened.

Biological safety cabinets should be installed in such a manner that fluctuations of the room supply and exhaust air do not cause the cabinet to operate outside its containment parameters.

### Aerosol-Generating Activities

Air-sampling studies have shown that many of the common manipulations of bacterial and viral cultures in research laboratories release aerosols of viable organisms. Aerosols can be generated by manipulation of liquids, tissue fragmentation, preparation of bacterial plates or the improper use of laboratory equipment including centrifuges, or breakage of containers with cell cultures. Aerosol-generating equipment should be placed towards the rear of the cabinet. Procedures with a potential for creating infectious aerosols or splashes may include:

– Centrifuging	– Pipetting
– Grinding	– Cell Sorting
– Blending	– Sonic Disruption
– Vigorous Shaking or Mixing	– Inoculating animals intranasally
– Opening containers of infectious materials	– Harvesting infected tissues from animals or embryonate eggs

### Safe Work Practices

#### Prior to Beginning Work

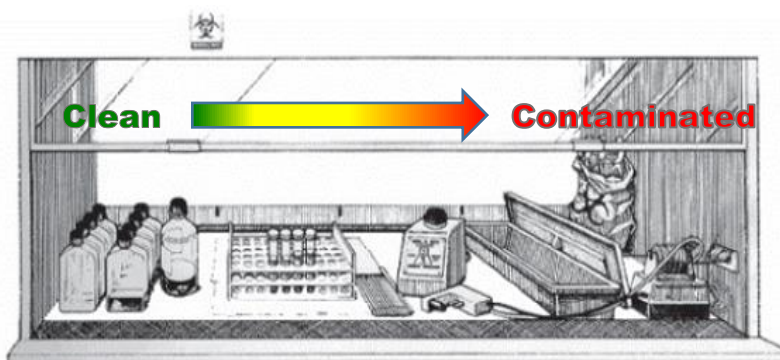
- Wear appropriate PPE.
- Remove all unnecessary equipment and supplies from the cabinet, as clutter alters airflow. Check that air grilles are clear.

## Working Safely in a Biological Safety Cabinet

- Turn on blower before using the BSC to remove particulates in the cabinet. Wait at least 5 minutes.
- Wipe down surface of cabinet interior with disinfectant.
- Place only items/equipment required for the immediate work inside the BSC to minimize the number of arm-movement disruptions across the air barrier of the cabinet.
- Materials should be surface-decontaminated with an appropriate disinfectant, particularly containers removed from a water bath.
- Place decontaminating solution inside the cabinet to facilitate quick cleanup of spills. Do not hang disinfectant bottle on outside grille of the BSC.
- Materials must not be block the front intake grille. All materials should be placed as far back in the cabinet towards the rear edge of the work surface as practical, without blocking the rear grille. Bulky items, such as biohazard bags, discard pipette trays, and suction collection flasks, should be placed to one side of the interior of the cabinet.

**Never place paperwork inside the biological safety cabinets.**

- Active work should flow from areas identified as clean across the work surface to those identified as contaminated areas as illustrated. Set supplies up accordingly.
- Adjust stool height so that your neck and face are above the sash opening.
- Ensure the drain valve under the work surface is closed so that all contaminated materials are contained within the cabinet should a large spill occur.

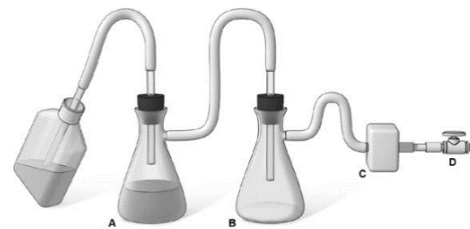


### During Work Activities

- All operations should be performed at least 4-6 inches from the front grille on the work surface.
- Arm movement in and out of the BSC should be slow and perpendicular to the front opening.
- Manipulations of materials should be delayed at least 1-minute after placing arms inside the BSC to allow the cabinet to stabilize, to "air sweep" the hands and arms, and to allow time for turbulence reduction.
- Open Flames and Bunsen burners are not recommended in the near microbe-free environment of a biological safety cabinet. Open flames create heat buildup and turbulence that disrupts the pattern of HEPA-filtered air being supplied to the work surface. **Alternatives:** *Small electric furnaces for decontaminating bacteriological loops or disposable loops can be used.*

## Working Safely in a Biological Safety Cabinet

- In-line HEPA filters must be used to protect all vacuum lines from contamination and fluid intake during aspiration. The left suction flask (A) is used to collect fluids into a suitable decontamination solution. The right flask (B) containing appropriate disinfectant serves as a fluid overflow collection vessel. An in-line HEPA filter (C) is used to protect the vacuum system (D) from aerosolized materials.



- Inactivation of aspirated materials can be accomplished by placing sufficient chemical decontamination solution into the flask to kill infectious agents, as they are collected. Once inactivation occurs, liquid materials can be disposed of as noninfectious waste.
- Horizontal pipette discard trays, which may contain an appropriate chemical disinfectant, should be used within the cabinet. Potentially contaminated materials should not be brought out of the cabinet until they have been surface decontaminated or placed into a closable waste container for transfer to an incubator, autoclave, or another part of the laboratory. The closable waste container should also be surface decontaminated prior to removal.
- Ultraviolet (UV) lamps should not be used as the sole disinfection method in a BSC. UV lamps should be cleaned regularly to remove any film that may block the output of the lamp. The lamps should be evaluated regularly and checked with a UV meter to ensure that the appropriate intensity of UV light is being emitted. Replace the bulb when the fluence rate is below 40 uW/cm<sup>2</sup>. Unshielded UV lamps must be turned off when the room is occupied to protect eyes and skin from UV exposure.

### After the Completion of Work

- Wipe down the surfaces of all containers and equipment with an appropriate disinfectant before removal from the cabinet.
- Leave the blower on for several minutes with no activity so that any airborne contaminants will be purged from the cabinet.
- Wipe down the cabinet interior with disinfectant.
- Remove gloves and wash hands.
- All non-liquid waste from the cabinet including used gloves shall be disposed of biological/regulated medical waste.

### **BSC Maintenance**

- The operational integrity of a BSC must be validated upon installation, annually, and after repair or relocation.
- Certification labels will be affixed to the BSC with the date certified and the recertification due date.
- BSCs are certified at a specified sash height. This height is indicated on the cabinet.
- A qualified technician must perform all BSC repairs. Any malfunction in the operation of the BSC should be reported immediately the PI/Faculty/Lab Supervisor.
- EHS will arrange for repair with a qualified technician.

### Contamination Prevention

- Disinfection & Decontamination of the area underneath the grille should be cleaned after a spill into the grille; on a regular basis depending on the type of work being conducted; and before filter changes or relocation. *Media is commonly splattered on the front grille, allowing fungus to grow undetected on the under surface of the grille.*
- Lab coat and sleeves can introduce contaminants into BSC and incubators. Use coats designated for working in the biological safety cabinet or tissue culture area, and launder frequently. Use disposable sleeve guards if contamination has been a problem.
- **Do not leave flasks of waste media in cabinet.** Properly inactivate waste and clean flask after each use. Waste media should not remain in flask overnight.
- Decontaminate the surface of carts or trays used to transfer culture flasks between the incubator and BSC or microscope.
- Keep pipette aids cleaned, especially the nosepiece, and replace filters regularly. Do not store pipette aids or carousel in the BSC.
- Disinfect and clean vacuum tubing.
- Do not alter a BSC in anyway such as taping items to the inside of the cabinet walls.
- **Do not store any items on top of or unnecessary items inside the BSC.** Disinfect and remove all items once the work is complete.

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### Classifications of Biological Safety Cabinets

BSC Class	Worker Protection	Product Protection	Environment Protection	Volatile Chemicals and Radionuclides	Nonvolatile Toxic Chemicals and Radionuclides
I	✓	✗	✓	When exhausted Outdoors <sup>1,2</sup>	Yes
II, A1	✓	✓	✓	Yes (small amounts) <sup>1,2</sup>	Yes (small amounts) <sup>2</sup>
II, A2	✓	✓	✓	When exhausted outdoors (small amounts) <sup>1,2</sup>	Yes
II, B1	✓	✓	✓	Yes (small amounts) <sup>1,2</sup>	Yes
II, B2	✓	✓	✓	Yes (small amounts) <sup>1,2</sup>	Yes
II, C1	✓	✓	✓	Yes (small amounts) <sup>1,2</sup>	Yes
III	✓	✓	✓	Yes (small amounts) <sup>1,2</sup>	Yes

1. Installation requires a special duct to the outside, and may require an in-line charcoal filter, and/or a spark-proof (explosion-proof) motor and other electrical components in the cabinet. Discharge of a Class I or Class II, Type A2 cabinet into a room should not occur if volatile chemicals are used.
2. A risk assessment should be completed by laboratory and safety facility personnel to determine amounts to be used. In all cases, only the smallest amounts of the chemical(s) required for the work to be performed should be used in the BSC. In no instance should the chemical concentration approach the lower explosion limits of the compounds.

<b>Laminar Flow Clean Bench / Clean Air Device (CAD)</b> <i>(This is NOT a BSC.)</i>	✗	✓	✗	<p><b>No</b></p> <p>These benches should never be used when handling cell culture materials, drug formulations, potentially infectious materials, or any other potentially hazardous materials.</p>
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