Working with Biological Safety Cabinets
Standard Operating Procedure

1 Introduction

This Biosafety Standard Operating Procedure (BSOP) outlines procedures and techniques for working safely with Biological Safety Cabinets (BSC). These procedures will ensure that Memorial University of Newfoundland (MUN) personnel are aware of the types of BSC, the requirements for BSC use and the proper techniques for safely working with BSC.

A properly maintained and certified BSC, in conjunction with good laboratory techniques, provides effective primary containment for work with infectious materials. The type of BSC determines whether it provides only protection for the operator, or protection for both the operator and the material in use i.e. whether or not it provides an aseptic environment. Product and operator protection is provided by a curtain of HEPA filtered air that is directed in a laminar flow pattern down from the top of the BSC and into grills at the back and front of the BSC. Contaminated air that is drawn in the front of the BSC and air contaminated within the BSC is HEPA filtered before being exhausted from the cabinet or recirculated onto the work surface.

Note that there are also "laminar flow cabinets/hoods/clean air hoods" that provide only product protection, but no operator protection. These are not BSCs (although they may look somewhat similar), so it is important to know the difference and be sure that you are using a BSC when operator protection is needed. Most often these laminar flow hoods have air blowing towards the operator, which is not the case with a BSC.

Also note that a fume hood is not the same as a BSC. More details about this are provided in this BSOP.

2 Scope

This BSOP applies to all MUN personnel performing procedures at containment level (CL) 2 that:

- produce infectious aerosols,
- involve high concentrations or large volumes of infectious materials, or
- are required as a condition of a biosafety certificate approved by MUN's Institutional Biosafety Committee (IBC).

At CL 2 requiring level 3 operational practices (referred to as CL2+), ALL manipulations of biohazardous agents must be performed in a certified BSC (unless alternative approval is granted by the IBC, as indicated on the biosafety certificate).

3 Responsibilities

This section outlines responsibilities within the university for the implementation of this BSOP.

a. Environmental Health and Safety (EHS)

- Review and amend this BSOP as necessary,
- Confirm that BSC in use are adequately tested and certified (annually and after any BSC movement).

b. Unit Heads

- Ensure that the requirements outlined in this BSOP are communicated to all applicable members of the unit/department,
- Ensure that the components of this BSOP and the applicable legislation are implemented in all facilities under the Head's authority.

c. Laboratory Supervisors/Principal Investigators

- Ensure that the correct type of BSC is used for the intended work,
- Consult with EHS regarding the purchase and placement of BSC in the laboratory,
- Ensure that BSC users receive safety training regarding best laboratory work practices and this BSOP prior to commencing work with BSC,
- Ensure that a BSC is adequately decontaminated prior to being moved and/or serviced,
- Ensure that a BSC is tested and certified to NSF Standard 49 annually, and after being moved.

d. Laboratory personnel (staff/students)

- Understand how the BSC works prior to commencing work with the BSC,
- Follow the procedures for safely working with a BSC, as outlined in this BSOP,
- Immediately notify your immediate supervisor of any BSC malfunctions. If this results in an exposure to biohazardous materials, immediately notify the Biological Safety Officer (BSO).

4 Definitions

Biological Safety Cabinet (BSC): A primary containment device that provides protection for personnel, the environment, and the product (depending on BSC class – see appendices), when working with biological material.

Clean air bench: Clean air benches do NOT protect the worker and are NOT BSCs. Clean air benches have HEPA filtered laminar air that flows over the product then out towards the worker. They provide a flow of clean air over the product and protect it from contaminants in the environment. A clean air bench provides product protection only. The worker is directly exposed to aerosols and particulates from the work. Clean air benches are not to be used for work with biohazard risk group 2 or higher material, hazardous volatile chemicals (or particulates), or radioisotopes.

Fume hood: an enclosed units from which gases, vapours and fumes are removed and discharged directly to the outside atmosphere. A fume hood is used to control the exposure of workers and lab occupants to hazardous chemicals and to prevent release of hazardous substances into the laboratory air.

HEPA filter: A device capable of filtering 99.97% of airborne particles 0.3 µm in diameter (which is particle size shown to pass through the filter easiest). Due to the effects of impaction, diffusion, and interception, HEPA filters are even more efficient at trapping and retaining particles that are either smaller or larger than 0.3 µm in diameter.

High concentration: Infectious material or toxins that are concentrated to a degree that increases the risks associated with manipulating the material (i.e., increases the likelihood or consequences of exposure).

Large volume: A volume of infectious material or toxins that is sufficiently large to increase the risk associated with the manipulation of the material (i.e., increases the likelihood or consequences of exposure or release). At MUN, this is considered anything equal to or greater than 10 litres of a biohazardous material in a single vessel (or in some cases, multiple vessels).

5 Types of BSC

The unique directional airflow in a BSC consists of:

1. HEPA-filtered air that passes over the work surface to provide a clean working environment;
2. an "air curtain" at the main opening of the BSC that prevents the mixing of potentially contaminated air from the laboratory with the HEPA-filtered air in the BSC;
3. continuous negative pressure in the BSC that is created by a continual draw of air into the BSC. This prevents the air inside from escaping into the laboratory;
4. the air that is exhausted out of the cabinet into the laboratory (if applicable) is HEPA-filtered to prevent release of particulate contaminants.

There are three classes of BSC available (refer to Appendix A for a summary table of key characteristics of Class II BSC, and Appendix B for diagrams of each).

Class I - provide operator and environmental protection but **no product protection**. Class I BSC are often used to enclose equipment (e.g. homogenizers) or for procedures where product protection is not a concern (e.g. cage changing). Class I BSCs can recirculate exhaust air

into the containment zone, or exhaust directly to the outside atmosphere when hard-ducted to the facility's heating, ventilation, and air conditioning (HVAC) system. Since the air is never recirculated within the BSC, it is possible to work safely with minute quantities of volatile toxic chemicals if the BSC is hard-ducted.

Class II - are designed for **personnel, environmental and product protection**. There are four types of class II BSC (types A1, A2, Bl, and B2) which differ by the ratio of air exhausted from the BSC to that which is recirculated within the BSC, and the type of exhaust system present.

Type A cabinets usually exhaust filtered cabinet air into the room, but are sometimes ducted out of the building by means of a "thimble connection."

- **Type Al** cabinets are never hard-ducted (i.e. never exhausted by a hard duct to the outside of the building) and are not suitable for work with volatile toxic chemicals or volatile radionuclides because 70% of the air is recirculated which could cause a dangerous buildup of the toxic materials inside the BSC, or inside the containment zone.
- **Type A2** cabinets are similar to type A1 cabinets; however, they have a greater inflow velocity and always have negatively pressured contaminated plenums or positively pressured contaminated ducts/plenums surrounded by negatively pressured ducts/plenums. This design feature ensures that the potential leaks in the positively pressured ducts or plenums are drawn inward rather than out into the containment zone. This type of BSC is suitable for work with minute amounts of volatile toxic chemicals and radionuclides, if ducted out of the building with a thimble connection.

Type B cabinets **are hard-ducted** through a dedicated duct exhausted to the atmosphere after passage through a HEPA filter.

- **Type Bl** is suitable for work with low levels of volatile toxic chemicals and trace amounts of radionuclides, as long as the work is performed towards the back of the cabinet where air is discharged directly to the outside atmosphere.
- **Type B2** draws room air into the top of the cabinet, through a HEPA filter, and then downwards over the work surface. The building exhaust system draws the air through the front and rear grilles into a contaminated plenum and then through a HEPA filter before being exhausted out of the cabinet directly to the outside atmosphere. These are suitable for work with volatile toxic chemicals and radionuclides because they are hard-ducted and the airflow within them is designed so that air is never recirculated within the BSC or within the containment zone.
    - Reversal of airflow from the face of a BSC, also known as a puff-back, can occur in Class II type B2 BSCs, for example upon failure of the HVAC system, power, or the exhaust fan serving the BSC. Every effort is to be made to address puff-backs mechanically (CBS Matrix 3.7).

Class III – are totally enclosed and gas-tight with HEPA filtered supply and exhaust air. Work is performed with attached long-sleeved gloves. They are designed for work with Risk Group (RG) 4 pathogens. None are in use at Memorial.

6 User Guidelines

a. Purpose and Use of the BSC

- BSCs are the primary means of containment for working safely with biohazardous agents. BSCs are designed to reduce the risk of infection by isolating the activities in the BSC from the laboratory environment.
- BSCs provide personnel protection and environmental protection by using a combination of directional airflow and a High Efficiency Particulate Air (HEPA) filter. Bacteria, spores and viruses are removed from the HEPA-filtered air.

b. Mechanical Operation of a Biological Safety Cabinet

- **Operation Panel and Main Features:** The functions of a BSC are controlled through a panel located on the front of the cabinet above or beside the sash (window). Switches on the panel control the lighting, the blower/fan system, the electrical outlets, and the alarm system bypass.
- **Magnehelic Gauge:** Users should routinely monitor the pressure on the magnehelic gauge so that you will know what it should be for your cabinet since the normal reading differs from one cabinet to another. The magnehelic gauge provides a "gross" or approximate indication of HEPA filter loading, i.e., the volume of particulate matter the filter has accumulated as the cabinet operates. It provides one indication of how rapidly the filter's capacity is being diminished. This is measured by reading either motor blower suction or pressure.
- **Blower:** The blower motor should be turned on at least 5 minutes prior to using the BSC. To maintain the proper airflow pattern, the sash (window) should only be opened to the height marked on the front panel (usually a maximum of 10 inches). Although the BSC may be turned off after decontamination as described under "Upon completion of work" (page 6), many users choose to leave the BSC running continuously to maintain a clean environment at all times. In addition, if a BSC is used many times during a day, it should be left running until all work is complete because frequent turning on and off of the BSC decreases the life expectancy of the motor.
- **Alarm:** There should be an alarm that will sound if the air circulation in the cabinet has been compromised (usually because the sash or window has been raised too far, but also for other reasons such as a blocked HEPA filter). A BSC must not be used when an alarm sounds or a warning light is on. An alarm must never be disengaged as it indicates improper airflow which affects the cabinet's performance and may endanger the researcher or the experiment.

  - On older BSCs that do not have alarms, it is particularly important to routinely check the magnehelic gauge to ensure that the air flow pressure remains constant.
- **Lighting:** There are two types of lighting inside the BSC: fluorescent or LED, and Ultraviolet (UV). The fluorescent or LED light should normally be ON while work is being done within the cabinet. Although the direct exposure to UV light (approximately 260 nm wavelength) can reduce the number of pathogenic microorganisms on exposed surfaces and in air, the routine use of UV lamps to decontaminate a BSC is not recommended for the following reasons.
  - UV light has poor penetrating power. UV irradiation is ineffective if a microorganism is protected by dust, dirt, or organic matter. A liquid chemical disinfectant should be the primary method of cleaning and disinfecting the interior of a BSC.
  - UV irradiation does not penetrate into cracks or through the grilles of a BSC.
  - UV irradiation can cause deterioration of various materials, including certain plastics and tubing.
  - the accumulation of dust, dirt, grease or clumps of microorganisms reduce its germicidal effects.
  - exposure to UV light is hazardous: it may result in severe eye damage and bums to the skin.
  - Nevertheless, UV lights may be useful in certain situations, if properly maintained, such as when using spore-forming bacteria since bacterial spores are resistant to chemical disinfection.
  - UV irradiation of the work area should only be used as a secondary method of disinfection in the cabinet. Never rely on UV irradiation alone to disinfect a contaminated work area.
  - The UV lamp should be routinely tested with a UV meter to verify that the proper intensity (i.e., 40 $\mu W/cm^2$) is being delivered at the appropriate wavelength (i.e., 254 nm) in the centre of the work area.
  - UV lamps must be turned off whenever the laboratory is occupied unless the sash can be lowered so that it is completely closed.
- **Outlets:** Electrical devices can be used inside the BSC by using the plugs provided.

7 Procedure for Safely Working with Biological Safety Cabinets

Planning: Thoroughly understand the procedures to be performed and the functions and limitations of the BSC that you are using. Identify/collect and place all of the necessary equipment in the BSC prior to beginning work.

- **Only one person should work in a BSC at one time** (even in 6 foot long BSCs). Two people will disrupt the airflow, creating turbulence that could result in biohazardous

aerosols coming out of the cabinet, or cause cross-contamination of work in the cabinet. i.e. it can be hazardous for you and/or your science.

- Large BSCs are appropriate for experiments that need lots of space, **not for two people to work at one time**.

Start-up

- Turn **OFF** the UV lamp (if it is on) and turn **ON** the fluorescent or LED light.
- Check that the sash is at the appropriate height. Adjust stool height so that the user's underarms are level with the bottom of the sash.
- Turn **ON** the blower (if it is off; many labs leave the blower running all the time) and allow it to run for at least 5 minutes before starting work in the cabinet.
- If present, test the airflow alarm and ensure it is switched to the "on" position.
- Routinely monitor the pressure on the pressure (magnehelic) gauge so that you will know what it should be for your cabinet since the normal reading differs from one cabinet to another. If the differential pressure drops by more than 0.2 then do not use the cabinet and call for service.
- Disinfect the interior surfaces with a disinfectant appropriate for the biohazardous agents used in the laboratory (e.g. 70% Ethanol (EtOH), Virox, an H_2O_2 disinfectant). **Note that bleach can corrode the stainless steel and make the BSC difficult to decontaminate so it should not be used for routine decontamination.** If bleach or other corrosive disinfectant is required (e.g. for spill clean-up) then be sure to thoroughly rinse the surface.
- Assemble all materials required for manipulation and load into the BSC.
    - Care should be taken not to overcrowd or block the front or rear grilles to prevent the appropriate airflow patterns from being compromised.
    - **Disinfectant effective against the agents in use should be placed inside the BSC** so that you do not have to reach out with contaminated gloves to get it.
    - Place **aerosol generating equipment** (e.g., vortex mixer, sonicator) towards the back of the BSC, without blocking the rear grille.
    - When there is significant **potential for splatter or splashes** to occur during manipulations of infectious material or toxins, the work area should be lined with a plastic-backed absorbent pad.
- After loading material in the BSC, allow sufficient time for the air to purge and the airflow to stabilize before initiating work. This will be specified in the manufacturer's instructions, and is generally 3-5 minutes.

Working in the BSC

- Wear protective gloves that cover the cuffs of lab coat sleeves to prevent contaminated air from entering the sleeve. Lab coats (or closed front gowns) with fitted cuffs rather than loose sleeves are recommended.
- Perform operations as far to the rear of the work area as reasonable. Ensure that elbows and arms do not rest on the grille or work surface.
- Movement of arms into and out of the cabinet can disrupt airflow, which can allow contaminants to enter or escape the BSC. Whenever possible, place all materials needed for a procedure inside the cabinet before starting. Move arms slowly and move straight out of the cabinet perpendicular to the front opening; do not sweep arms across the front of the cabinet. Do not walk quickly in front of a cabinet when someone else is working.
- Place supplies, equipment and papers well back from the front of the cabinet, positioned so that air intake or exhaust grills are not obstructed.
    - Never put anything on the grill at the front opening of the cabinet.
    - Do not block the air openings/grill at the back of the cabinet.
- Segregate non-contaminated ("clean") items from contaminated ("dirty") items. **Work should always flow from "clean" to "dirty" areas.**
- Material should be discarded in a waste container located **inside** and towards the rear of the cabinet workspace. Do not discard materials in containers outside of the cabinet.
- Before using vacuum aspiration, verify that there is fresh bleach in the collection flask (final concentration when the flask will be full of at least 0.5% hypochlorite in the collection flask (i.e. 10% final dilution of stock bleach).
- **Clean up spills** as soon as they occur. Remove and disinfect the grill if contaminated and remember to clean under the grill (follow procedure outlined in BSOP-03).
    - If the spill was relatively large or contained concentrated infectious material then allow the cabinet to sit undisturbed for at least 5 minutes for aerosols to clear before beginning cleanup.
    - While you wait remove and disinfect or dispose contaminated personal protective equipment (PPE) and wash thoroughly.
    - Then don clean PPE and proceed with spill cleanup and decontamination of interior surfaces, including the window/sash.
    - Remember to allow appropriate contact time for the disinfectant.

Upon completion of work

- Upon completion of work, allow sufficient time for the air in the BSC to purge (i.e., pass through the filter) before disrupting the air curtain by removing hands or unloading material from the BSC. The purge time will vary by model and can be up to several minutes.

- Surface-disinfect objects before removal from the cabinet. Remember that aerosols generated during operations in the cabinet such as pipetting might have contaminated objects in the cabinet, so there does not have to have been a spill for this step to be necessary.
- Disinfect the interior surfaces of the BSC, including sides, back, lights, and interior of the glass, with a disinfectant effective against the pathogens in use, allowing an appropriate contact time (CBS Matrix 4.6). If a corrosive disinfectant is used, the surface should be thoroughly rinsed with water after disinfection to avoid corrosion of the stainless steel surfaces.
- Periodically remove the work surface and disinfect the area beneath it (including the catch pan) and wipe the surface of the UV light with disinfectant.
- If appropriate turn off the blower and fluorescent or LED lamp, and turn on the UV light if appropriate (see section 6B, Lighting).
- Disinfect or dispose of personal protective equipment appropriately and wash hands.

Warnings

- Equipment creating air movement (e.g., vacuum pumps, centrifuges) may affect the integrity of the airflow and should not be used within the BSC.
- Windows that open should be kept closed when the BSC is in use.
- Work in a BSC should only be conducted by one person at a time (even in a large BSC).
- Sustained open flames are **prohibited** within a BSC. On-demand open flames (e.g., touch-plate microburners) are to be avoided as they create turbulence in the BSC, disrupt airflow patterns, and can damage the HEPA filter (CBS Matrix 4.6). **Non-flame alternatives (e.g., microincinerator, or sterile disposable inoculation loops) should be used whenever possible.**
- The HEPA filters in the BSCs remove particulates from air, but they are not effective at collecting chemical gases or vapours. If you need to use such material in a BSC, contact the Biosafety Officer for advice.
- Routine use of UV lamps to decontaminate a BSC is not recommended (see section 6B, Lighting).
- DO NOT use the BSC if the ALARM sounds or if there are other indications of cabinet malfunction such as no airflow, reduced pressure on magnehelic gauge (drop > 0.2), or unusual noises.

  If alarm or other indication of failure happens while using the cabinet:

  - Seal, surface decontaminate and remove any biohazardous material.
  - Decontaminate the interior of the BSC.
  - Switch off the alarm or the power if the motor is making noise.

  - Place a sign on the cabinet to indicate that it is broken and must not be used.
  - Contact Environmental Health and Safety for advice and servicing.
- If personnel may have been exposed to infectious material due to cabinet failure, then the supervisor must be promptly notified and an incident report completed and the appropriate first aid and medical follow-up action taken (refer to the post-exposure response in MUN's Biological Safety manual, section 4.3).
- To help protect the fragile air curtain at the front of the cabinet, BSCs must be located as far as possible away from areas where airflow patterns may be disrupted e.g., room air supply and exhaust grilles, doors, open windows, high traffic areas, and large pieces of equipment that generate heat (CBS Matrix 3.7.6).
- Consult EHS if your BSC appears to be located in an area where the air curtain may be disrupted. EHS can do a smoke test on the air curtain, and propose remedial actions.

8 Maintenance/Certification of Biological Safety Cabinets

When used for biosafety level 2 containment, BSC performance must be tested and certified (as specified in CBS Matrix 5.1):

- upon initial installation in the laboratory,
- annually thereafter,
- when moved from one area to another within the same room, or from one room to another,
- whenever maintenance is carried out on internal parts, and whenever filters are changed.

In biosafety level 1 laboratories, biological safety cabinets are often used primarily for product protection rather than personnel protection. Therefore, for biosafety level 1 containment laboratories, the testing and certification of biological safety cabinets noted above is recommended but not mandatory.

To arrange BSC maintenance or testing and certification, contact Technical Services.

Version History:

Version	Date	Author(s)	Notes
1.0	2021-03-10	Rod Hobbs	First writing.

Appendix A: Characteristics of BSC types (copied from the Canadian Biosafety Handbook, 1st Edition, 2016, Chapter 11).

	Type A1	Type A2	Type B1	Type B2
Minimum average inflow velocity through front opening	0.38 m/s [75 fpm]	0.51 m/s [100 fpm]	0.51 m/s [100 fpm]	0.51 m/s [100 fpm]
Air patterns	30% of the air is exhausted out of the BSC and 70% of the air is recirculated within the BSC	30% of the air is exhausted out of the BSC and 70% of the air is recirculated within the BSC	>50% of the air is exhausted out of the BSC and <50% of the air is recirculated within the BSC	100% of the air is exhausted out of the BSC
HEPA-filtered downflow air	Composed of mixed downflow and inflow from common plenum	Composed of mixed downflow and inflow from common plenum	Inflow air	Drawn from the containment zone or from the outside atmosphere
HEPA-filtered exhaust air	Recirculated to the containment zone or directly to the outside atmosphere	Recirculated to the containment zone or directly to the outside atmosphere	Exhausted through dedicated exhaust plenum to the outside atmosphere	Exhausted through dedicated exhaust plenum to the outside atmosphere
Type of exhaust	Can be thimble connected	Can be thimble connected	Hard-ducted	Hard-ducted
Contaminated ducts and plenums	Negatively pressured or surrounded by negatively pressured ducts or plenums; plenum may be positively pressured in some models	Negatively pressured or surrounded by negatively pressured ducts or plenums	Negatively pressured or surrounded by negatively pressured ducts or plenums	Negatively pressured or surrounded by negatively pressured ducts or plenums
Work with volatile toxic chemicals and radionuclides	No	Minute amounts if exhausted through thimble connection	Low levels of volatile toxic chemicals and trace amounts of radionuclides	Yes

Appendix B: Figures of BSC types (copied from the Canadian Biosafety Handbook, 1st Edition, 2016, Chapter 11).

Figure 11-2: Illustration of a Class II Type A1 Biological Safety Cabinet (BSC) (with a Positively Pressured Contaminated Plenum)

Cabinet exhaust may be recirculated into the room or vented to the outside atmosphere through an air gap type (thimble) connection, as shown. Purple shading indicates positively pressured contaminated plenum.

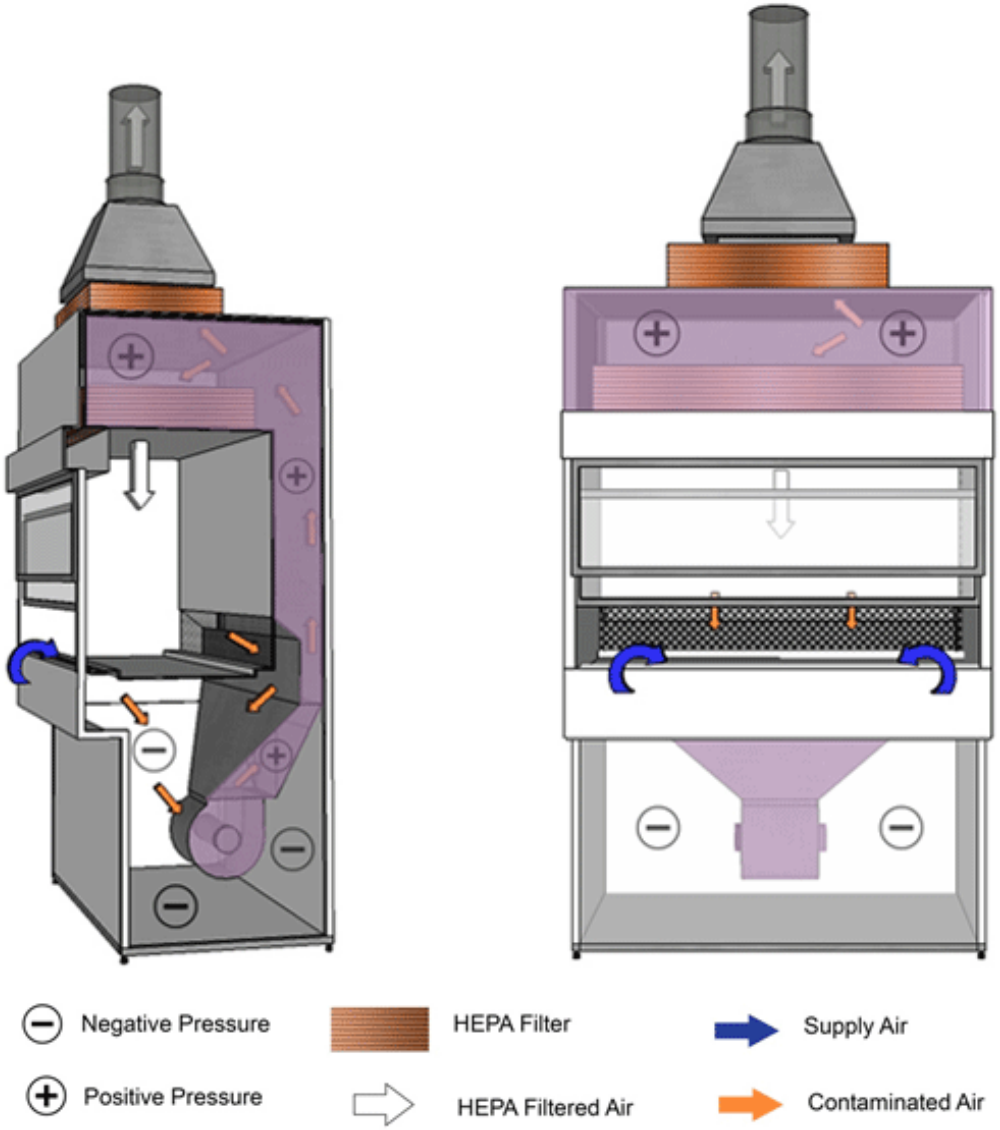


Figure 11-3: Illustration of a Class II Type A2 Biological Safety Cabinet (BSC)

Cabinet exhaust may be recirculated into the room or vented to the outside atmosphere through an air gap type (thimble) connection, as shown. Cabinet shown has a negatively pressured plenum.

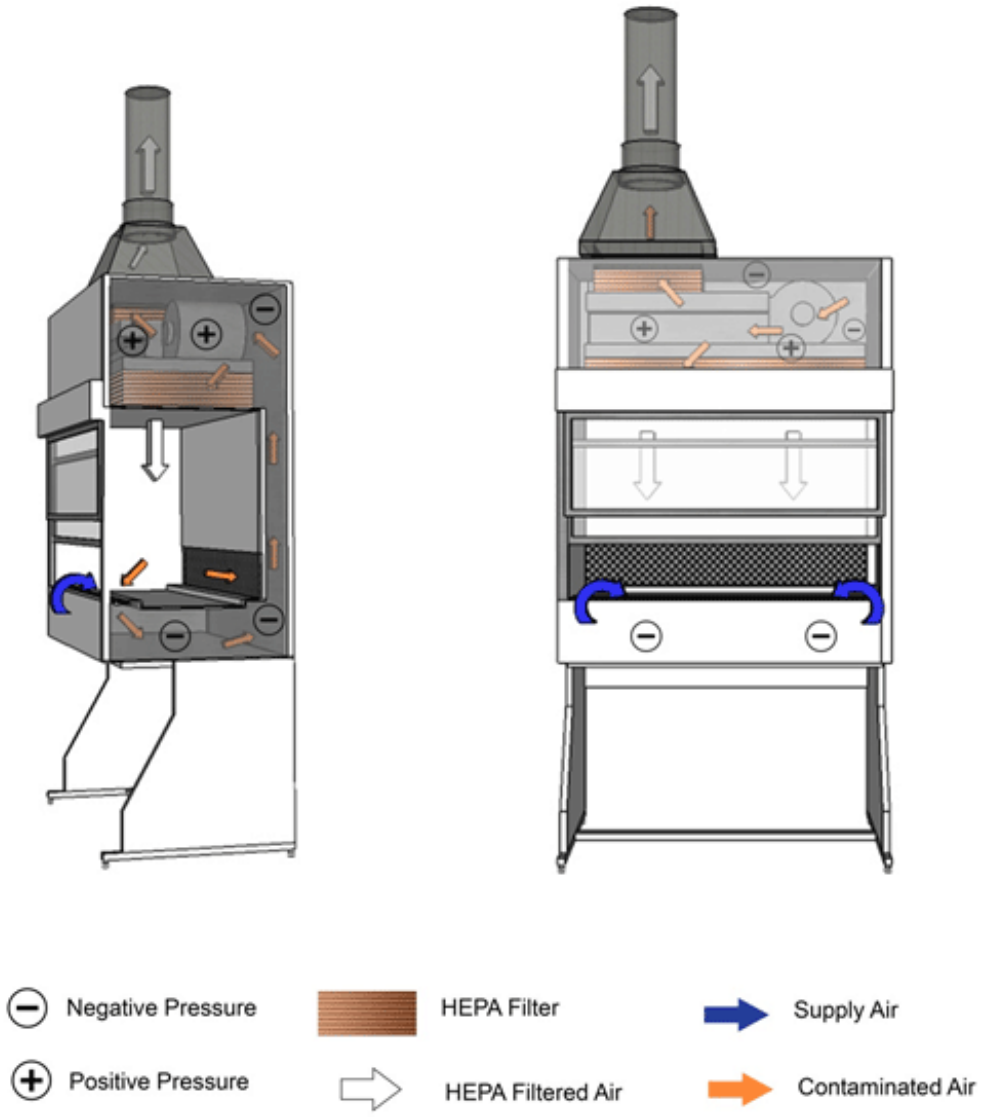


Figure 11-4: Illustration of a Class II Type B1 Biological Safety Cabinet (BSC)

Cabinet is vented to the outside atmosphere through a hard-ducted connection, as shown. The positively pressured plenum in this example is not contaminated, as the air is filtered before passing through the exhaust blowers.

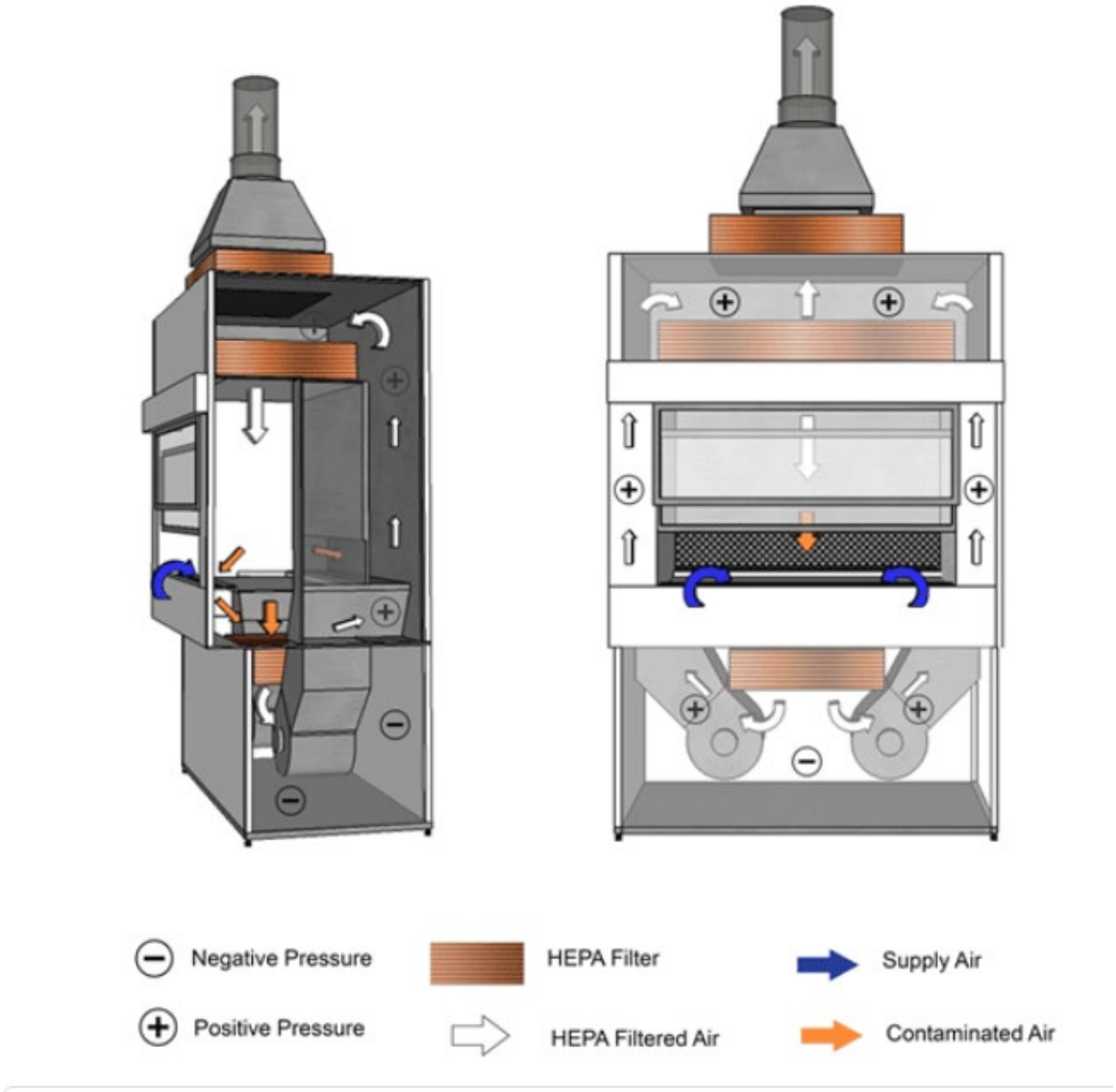
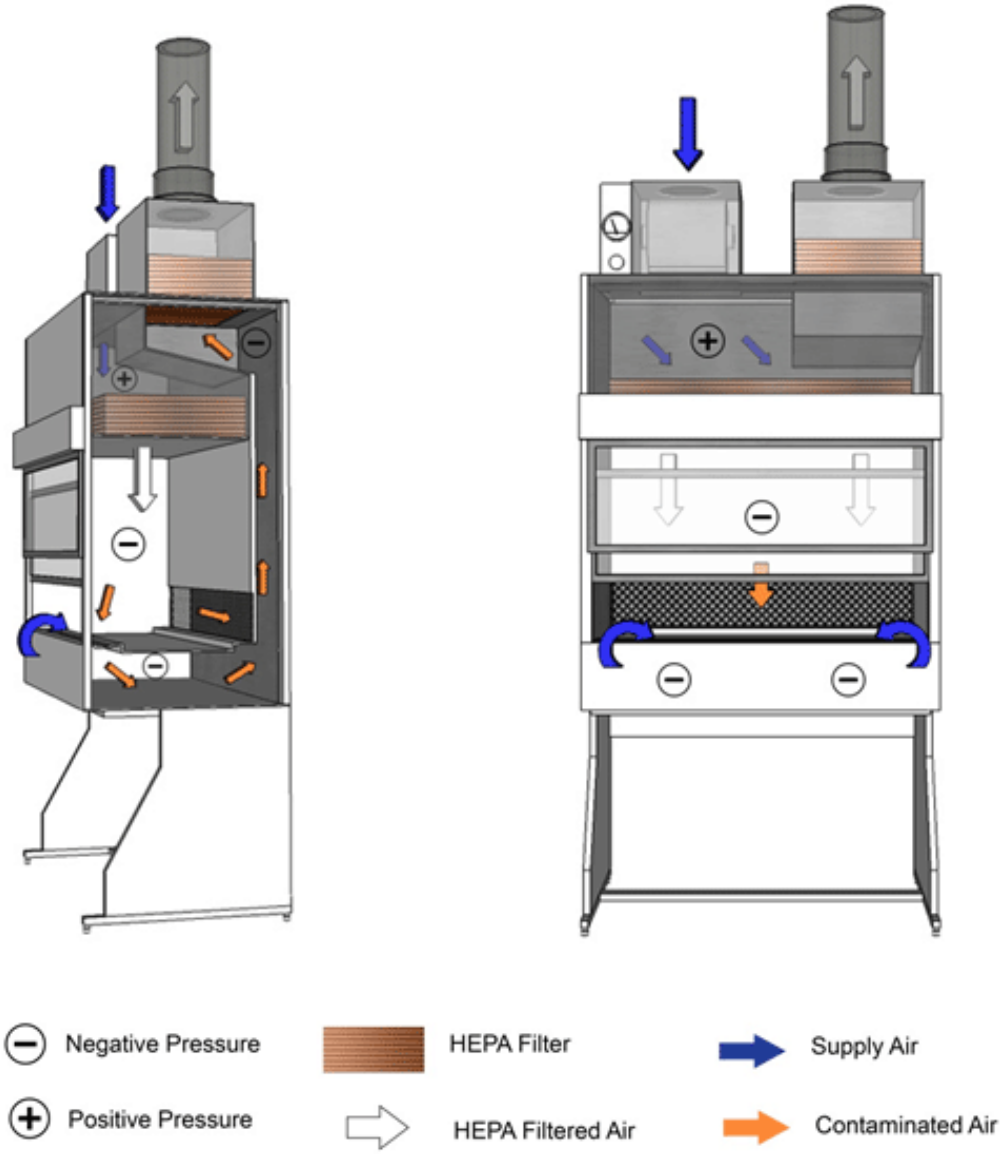


Figure 11-5: Illustration of a Class II Type B2 Biological Safety Cabinet (BSC)

Cabinet is vented to the outside atmosphere through a hard-ducted connection, as shown.



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1.0	2021-08-17	Rod Hobbs	First writing. IBC approval