Biosafety Cabinets

Definition:
A BSC is an enclosed, ventilated laboratory workspace for safely working with materials contaminated with (or potentially contaminated with) pathogens. A HEPA (high-efficiency particulate air) filter is used to remove particles (such as bacteria, viruses, pollen, etc.) from the cabinet's interior.

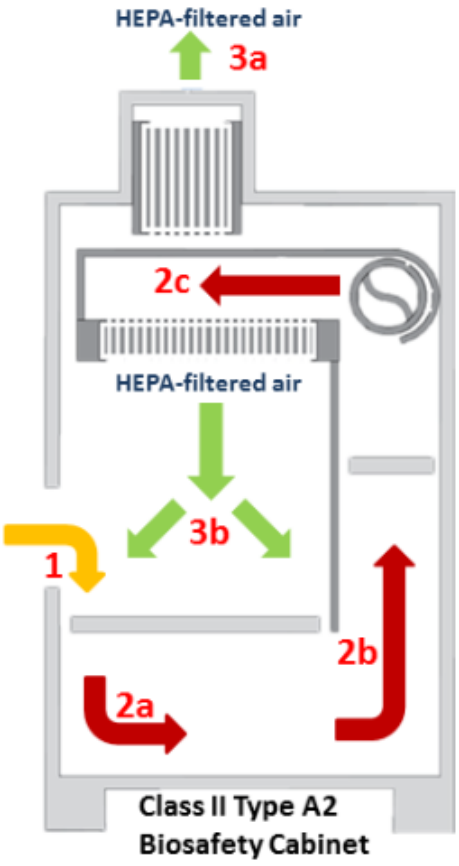
Purpose:
Class II A2 biosafety cabinets provide three basic types of protection:

a) Personnel protection from harmful agents inside the cabinet.
b) Product protection to avoid contamination of the work, experiment, or process.
c) Environmental protection from contaminants contained within the cabinet.

Biosafety cabinets **are not for chemical use** because 30% of the circulated air is exhausted into the room. HEPA filters do not offer fume protection.

How they work

1. The Class II, Type A2 BSC: An internal fan draws sufficient room air through the front grille to maintain a minimum calculated or measured average inflow velocity of at least 75 lfm (linear feet per minute) at the face opening of the cabinet. The supply air flows through a HEPA filter and provides particulate-free air to the work surface. Airflow provided in this manner reduces turbulence in the work zone and minimizes the potential for cross-contamination.
2. The downward moving air "splits" as it approaches the work surface; the fan draws part of the air to the front grille and the remainder to the rear grille. Although there are variations among different cabinets, this split generally occurs about halfway between the front and rear grilles and two to six inches above the work surface.
3. The air is drawn through the front and rear grilles by a fan pushed into the space between the supply and exhaust filters. Due to the relative size of these two filters, approximately 30% of the air passes through the exhaust HEPA filter and 70% recirculates through the supply HEPA filter back into the work zone of the cabinet. Most Class II, Type A2 cabinets have dampers to modulate this division of airflow.
4. A Class II Type A2 BSC is not to be used for work involving volatile toxic chemicals. The buildup of chemical vapors in the cabinet (by recirculated air) and in the laboratory (from exhaust air) could create health and safety hazards as 30% of the recirculated air is exhausted back into the laboratory.



Air Flow Pattern

1. Dirty room air is drawn into the front of the cabinet and mixes with contaminated air from the chamber

2a. Contaminated air is pushed below the work surface

2b. Contaminated air is drawn up through the plenum

2c. Contaminated air is pushed through HEPA filters by cabinet blower motor

3a. 30% of HEPA filtered air leaves top of cabinet and recirculates into room or is removed by canopy exhaust

3b. 70% of HEPA filtered air enters cabinet from above and flows down on work surfaces under unidirectional flow.

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Working in a BSC

Wear appropriate personal protective equipment. Lab coat should be buttoned over street clothes and gloves should be worn to provide hand protection. Additional PPE may be used depending upon the outcome of a risk assessment.

Check certification sticker to confirm that BSC has been certified within the past 12 months.

Operate cabinet blowers for at least 5 minutes prior to starting work.

Disinfect cabinet surface prior to starting work.

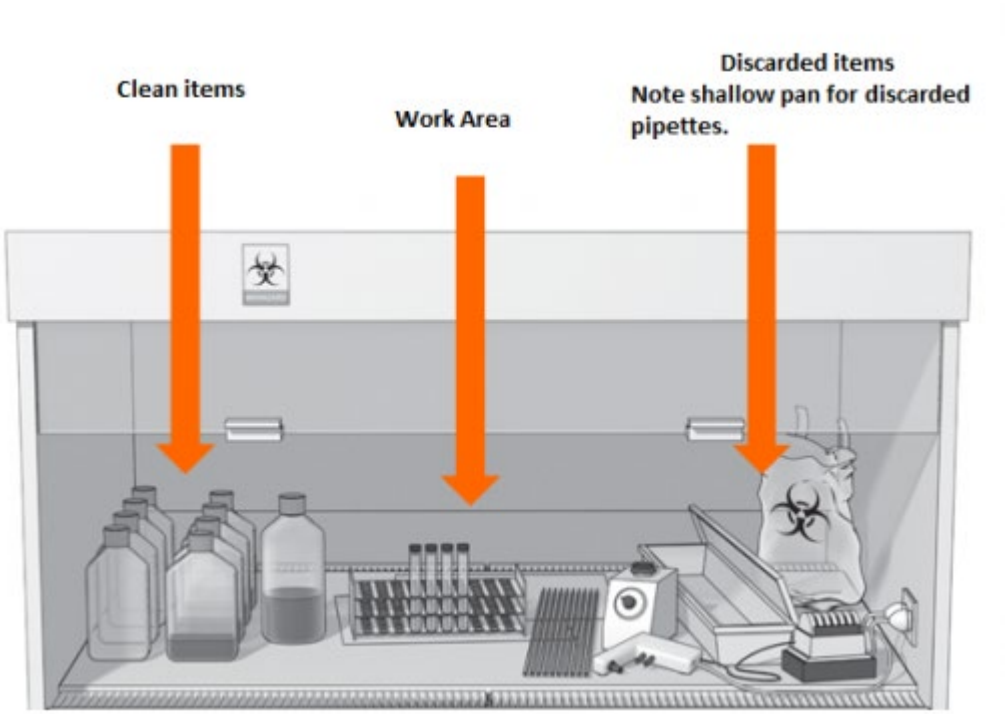
Set the sash at the correct height, 8” to 10” depending on manufacturer’s guidelines.

Close the drain valve under the work surface prior to beginning work so that all contaminated materials are contained within the cabinet should a large spill occur.

Before beginning work, the investigator must adjust the stool height so that their face is above the front opening.

Set up your cabinet to reduce potential for contamination. All materials must be placed as far back in the cabinet as practical, toward the rear edge of the work surface and away from the front grille of the cabinet.

Active work should flow from the clean to contaminated area across the work surface.



Aspirator suction flasks must contain an appropriate disinfectant, and a High Efficiency Particulate Air (HEPA) in-line filter. See: Vacuum systems below.

All operations should be performed on the work surface at least four (4) inches from the inside edge of the front grille.

Open flames (i.e., Bunsen burners) are rarely necessary in the near microbe-free environment of a biological safety cabinet and are an artifact left over from usage older model cabinets that only provided personnel protection.

Poster: Create a Safe Working Environment in Your Biological Safety Cabinet

Spills/Decontamination

Spills:

1. The lab should have components readily available to address spills: absorbent materials, disinfectant (e.g., 10% freshly prepared bleach), tongs or forceps to pick up broken containers and a biohazard-waste container.

2. Wear the approved PPE designated for your laboratory. At a minimum, laboratory coats should be worn buttoned over street clothing, and protective eyewear should be on at all times. In addition, nitrile gloves are necessary when handling culture, contaminated surfaces or equipment.
3. When a spill of biohazardous material occurs within a BSC, cleanup should begin immediately, while the cabinet continues to operate. Keeping the cabinet on will prevent the escape of airborne contaminants and ensure that whatever is in the cabinet stays in the cabinet, protecting those around you and the laboratory.
4. Before addressing the spill, first remove the tubes, pipettes and any other items that might have contained the spilled liquid and place them into the sharps or biohazard container within the cabinet. It is important to contain the contaminated materials inside the operating cabinet to avoid exposure to the laboratory. To prevent personal injury, always use tongs or forceps for picking up glass and sharps.
5. Cover the spill inside the BSC with absorbent material, such as paper towels, and let the spill soak in. This helps prevent aerosolization of the contaminant. Working inside the BSC, place the contaminated materials within a bag and seal the bag. Remove the bag from the BSC and place into the biological waste container. Apply appropriate disinfectant for the type of spill onto the surfaces, working from the outer edge to the middle of the spill's footprint. Applying the disinfectant from the outside to the inside of the spill helps trap any biological material.
6. Depending on what material was spilled and what disinfectant you are using, you might need to vary the disinfectant reaction time. As a rule of thumb, 00 minutes should be adequate to neutralize the contaminant.
7. After the spill has been contained and the disinfectant has had adequate time to react, use the towels to wipe up excess liquid. Place used towels into a waste container for disposal.
8. Apply disinfectant to the spill area again and give it appropriate time to work before wiping up with fresh towels. This helps ensure that all contaminated materials and surfaces are decontaminated. Also check the spill pan under the work surface and disinfect following the same procedure, if needed. Do not forget to disinfect the vertical (walls and sash) surfaces as well.
9. If bleach (or any other corrosive disinfecting agent) was used to clean the spill, use sterile water to rinse and then again to wipe the residual bleach (or disinfectant) from the working surface. Bleach is very corrosive to stainless steel and will cause damage, over time, if used to clean the cabinet.
10. After the cabinet has been cleaned, remove and properly dispose gloves and other protective equipment that has come in contact with contaminated material. Thoroughly wash your hands with soap and water. Run the BSC for at least 10 minutes before resuming work. Report the spill incident to your supervisor.

Following these steps will help you keep yourself and those around you safe if a spill in the BSC occurs. It will also help to maintain your equipment for years of use.

Gas decontamination

Gas decontamination of a BSC is performed to render it non-infectious and is achieved by exposing the work surfaces, exhaust filters, surfaces of the air plenums, and the fan unit to formaldehyde or hydrogen peroxide gas. Due to the potential for exposure to biohazardous agents and the chemicals used, this *may only be performed by a certified contractor* and is coordinated through the Biosafety office.

Certifications

Annually

Class II A2 biosafety cabinets should be certified annually. NSF International supports a consensus standard (NSF/ANSI Standard 49) addressing Class II BSC design, construction, and performance, which recommends certification at least annually.

When moving a biosafety cabinet

It is not an unusual practice to move permanently installed BSCs to other locations within a laboratory or to other laboratories. There are certain conditions that must be met prior to moving this equipment. BSCs should not be moved without consultation with a biosafety officer.

Existing BSCs and ancillary equipment, such as canopy connection exhaust ducting, electric and vacuum connections, should be cleared by a biosafety officer prior to disassembly. Depending on circumstances of the move, (i.e., cabinet use, new location, etc.), BSCs may be required to be contact and/or gas decontaminated. After a BSC is moved, it should be certified according to applicable performance standards.

After repairs

Some repairs will require that the BSC be certified once the repairs are completed. Examples: motor installation, filter changes, changes made to the plenum, etc.

Tissue culture practices

When working with valuable cell cultures, contaminating microorganisms, especially mycoplasma species, can be detrimental to the accuracy of resulting data, while introducing a potential hazard to lab personnel. Fortunately, the biological safety cabinet (BSC) provides a ventilated sterile work environment in which to safely handle biological samples, protecting both the cultures and users from hazardous particles.

It's important to remember although the filters and air barrier work to maintain clean airflow in the Class II BSC work area, they can't completely eliminate the risk of airborne or surface contamination. This can affect cells and threaten the safety of lab workers. Many cell cultures are costly and difficult to replace, so a major priority is the prevention of contamination. As such, there are several best practices that can be followed to ensure cellular viability is maintained, while saving on time, money and effort.

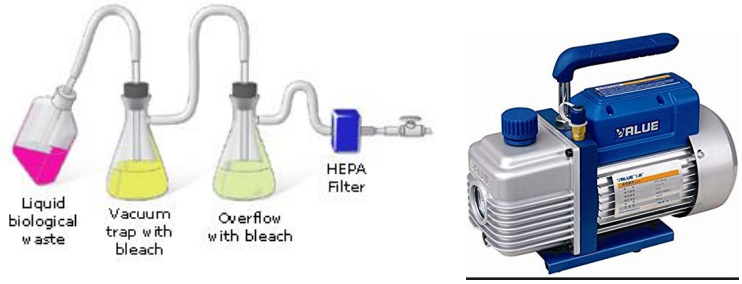
1. The cabinet should ideally be positioned in an area with low air turbulence, such as at the rear of the room. By locating it away from the entrance, the BSC is removed from exposure to major air currents, which are more likely to carry pathogens.
2. Users should ensure all necessary supplies and materials are wiped down and placed in the workspace at the beginning of each session, and refrain from removing waste or other materials until the work is finished or covered, so that the potential for contaminants being introduced to the working environment can be reduced.
3. Work "clean-to-dirty" as you gather supplies on the left, process the work in the middle and discard waste to the right. This also facilitates fast cleanup of spills to eliminate this route of contaminating the cultures, as well as the surrounding environment.
4. The cabinet's airflow at the front opening is essential to preventing pathogens from entering the surrounding air, so it is important that there are no obstructions to the opening of the BSC. Cabinets

are commonly fitted with over-the-grille armrests to avoid such obstructions while working within the cabinet.

5. By establishing good cleaning and sterilization protocols, labs can prevent contamination in an efficient and reliable way. A good practice to follow before and after your culture work is to wipe the work surface (as well as vertical surfaces)and materials placed in the cabinet with 70% ethanol, a 10% dilution of bleach or other appropriate disinfectant. After using bleach, surfaces should also be wiped with sterile water or 70% ethanol to remove any residual chlorine, which if left could corrode stainless steel surfaces. Always be sure that the water used is sterile, as wiping with non-sterile water may cause recontamination of the cabinet surfaces.
6. Use personal protective clothing (lab coat, gloves and eye protection) to avoid the hazards associated with the experiment. In order to minimize the potential for contamination from human sources, it's always recommended that users wash their hands thoroughly before putting on gloves for any handling of cultures. This removes bacteria, fungi and microscopic dead skin particles, which can be a potential source of contamination. Do not rinse gloves with ethanol (antiquated practice). Ensure that bare skin is covered to avoid contamination to the skin as well as the user's skin causing contamination of cell cultures. Ensure that the user is correctly positioned with their face above the front opening, not only to provide ergonomic conditions, but also to position the face and body correctly to minimize contamination.
7. The Centers for Disease Control and Prevention states that a BSC must be routinely inspected and tested by trained personnel, following strict protocols, to verify that it is working properly. Certification is performed annually, and whenever a cabinet is moved.
8. In case of a spill, ensure that the BSC is properly decontaminated.

<u>Vacuum/aspiration systems</u>

Protection of vacuum systems from biological exposure is an important facet of biocontainment. Please see the image below. The first flask (pink fluid) is the contaminated material that is being aspirated. The next suction flask (yellow fluid) is used to collect the contaminated fluids into a suitable decontamination solution (concentrated bleach). The next flask is the fluid overflow collection vessel. The in-line HEPA filter is located between the overflow flask and the connection to the vacuum pump.



<u>Repairs</u>: EH&S can assist you with contact information for certified repair personnel.

<u>Removal from service:</u> EH&S can assist you with contact information for certified decontamination personnel as well as disposal procedures.

The Effectiveness of HEPA filters on DNA: White Paper

Three of the most common pitfalls regarding the use of **ultraviolet light** in biosafety cabinets: Baker

Bunsen burner alternatives

- Fireboy™ by Integra Sciences
- Bacti-cinerator IV™
- Electric Bunsen burner
- FlameBoy™ portable flame sterilizer
- Glass bead sterilizer