

Class II Biosafety Cabinets

Introduction

Class II biosafety cabinets (BSCs) are primary engineering controls typically used for microbiological studies, cell culture work, pharmaceutical procedures, and toxicology studies. BSC classifications and standards for the United States are set by NSF International (formerly the National Sanitation Foundation). Like its predecessor, [NSF/American National Standards Institute \(ANSI\) Standard 49:2018](#)—titled Biosafety Cabinetry: Design, Construction, Performance, And Field Certification—provides information relevant to certifiers, end-users, facilities managers and others in the field of biosafety. This standard defines five types of Class II cabinets (A1, A2, B1, B2 and C1) that are distinguished by differences in airflow patterns and velocities, high-efficiency particulate air (HEPA) filter/ultra-low particulate air (ULPA) filter positions, ventilation rates and exhaust methods.

When properly maintained and used in conjunction with good laboratory techniques, BSCs provide effective primary containment for work with human pathogens. In containment level 2 facilities, BSCs are used for procedures with the potential to produce infectious aerosols and for high concentrations or large volumes of infectious material. Every person working in a BSC needs to be trained in its correct use and have a good understanding of how the cabinet works.

Class II BSC types A1 and A2 are typically used for work in biosafety levels (BSL) 1 through 3. Because type A1 cabinets are not suitable for work with chemicals, use of type A2 cabinets is more prevalent. When vapors are not hazardous and will not interfere with the work when recirculated, it is acceptable to use an A2 cabinet with a small amount of chemicals when the cabinet is exhausted to the outdoors for removal of gases.

Type B1 and B2 cabinets are also typically used for BSL 1 through 3. As with type A2 cabinets, type B1 cabinets can be used for work generating chemical vapors when the vapors do not interfere with the work when recirculated or when the work is done in the directly exhausted portion of the cabinet. The type B2 total exhaust cabinets are widely used in toxicology laboratories and similar applications where chemical effluent is present and clean air is essential.

Class II, Type C1 is the first BSC that can be used in a recirculating Type A-mode for standard microbiological work or can be connected to an exhaust system to function in Type-B mode for handling hazardous chemical vapors or radionuclides. Type C1 enclosures provide protection by filtering hazardous particulates and aerosols, such as agents that require BSL 1 through 3 containments, with the option of venting away hazardous fumes.

All types of Class II cabinets may be used in BSL 4 laboratories when workers utilize positive pressure suits.

Class II BSCs give:

- Personnel protection from harmful agents used inside the BSC
- Product protection to avoid contamination of the work, experiment, or process from outside contaminants
- Environmental protection from contaminants contained within the BSC

Class II BSCs key features are:

- A front access opening with carefully maintained inward airflow
- HEPA / ULPA-filtered, vertical, unidirectional airflow within the work area
- HEPA / ULPA-filtered exhaust air to the room or exhaust to a facility exhaust system

Class II Biosafety Cabinet – Type A2

All BSCs at UTA are Class II Type A2 cabinets. Currently, as of May 2023 there are 66 BSCs actively used by the following domains: Animal Care Facility (ACF), Bioengineering, Biology, Chemistry, Chemistry & Biochemistry, College of Nursing and Health Innovation (CONHI), Kinesiology, Material Science & Engineering, North Texas Genome Center (NTGC), Physics, Psychology, and Shimadzu Institute for Research Technologies (SIRT).

When planning to have a new BSC installed and looking the best place for it, it is good to take into consideration the information in [NSF/ANSI 49-2018 Annex E](#) concerning the clearances around the cabinet. When a new BSC arrives, it should be inspected carefully. The invoice should be compared with the delivered equipment. If there are any damages or missing materials, these should be reported immediately to the proper carrier and the BSC supplier regardless of how insignificant they may appear at first. Cabinet should be positioned so that there is a 6" minimum clearance behind and on each side of BSC. Also, it is advisable to avoid installing the BSC too near air outlets or fume hoods. These can affect the airflow pattern of the BSC.

[Certification of the BSC needs](#) to be arranged after it has been installed. Facilities Management personnel may be needed to connect the BSC to laboratory plumbing, electrical, and/or supply/exhaust air ventilation systems.

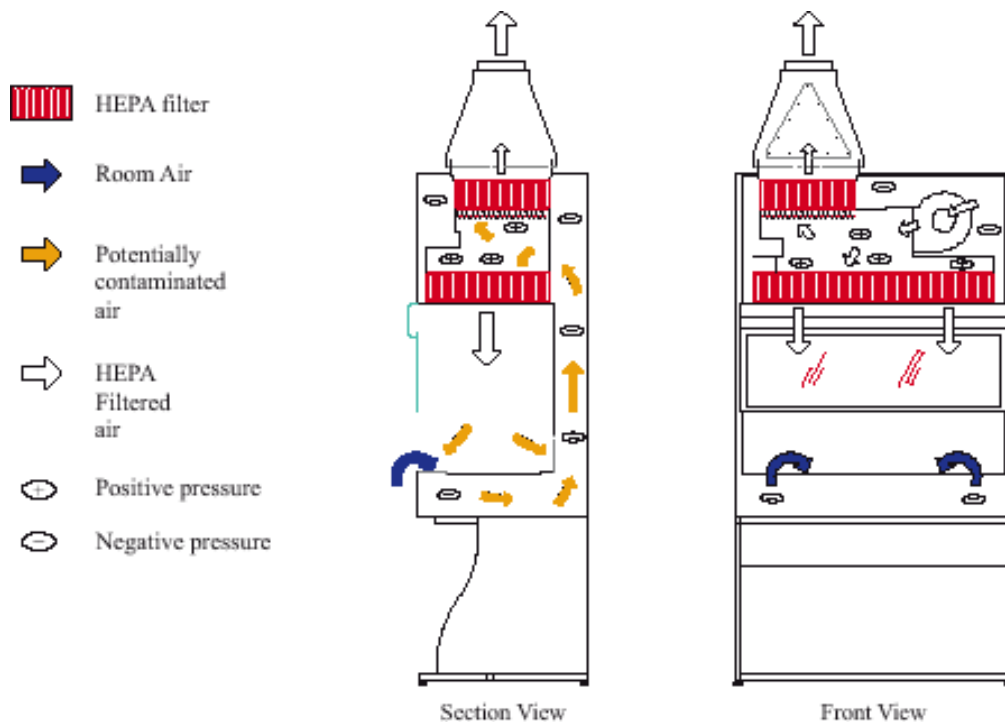
The airflow characteristics of the Class II Type A2 BSC are:

- Recirculating system
- May be vented into the room or to the facility's HVAC system through a canopy exhaust connection
- Remaining air is recirculated to the work area through a HEPA / ULPA supply filter
- HEPA- / ULPA-filtered down flow air is a mixture of recirculated and inflow air from a common plenum, and will vary in total volume based on the cabinet design
- Intake air velocity for a Type A2 is a minimum of 100 feet per minute (FPM)
- All biologically contaminated ducts and plenums are under negative pressure or surrounded by negative pressure ducts and plenums

The basic characteristics of Class II Type A2 BSC are:

Class II Biosafety Cabinet		Type A2	
Containment and protection	Provides protection from particulates	Personnel	Yes
		Product	Yes
		Environment	Yes
	Provides protection from vapors & gases in adjunct to microbiological work	Personnel	Only if exhausted to facility exhaust system
		Product	No
		Environment	Only if exhausted to facility exhaust system
Airflow characteristics	Cabinet face velocity		Minimum of 100 FPM
	Recirculated		App. 70 %
	Exhausted		App. 30%
Exhaust characteristics	Cabinet exhaust destination	To room	Yes
		Vented outside	Optional
		Connection type	Canopy
	Cabinet exhaust source		Common plenum
Plenum	Biologically contaminated plenum pressure		Negative to room or surrounded by negative pressure

The picture below shows the room air, contaminated air, and HEPA-filtered air movements in a Class II Type A2 BSC.



In October of 2015, NSF International issued a memorandum to field certifiers of the NSF/ANSI 49 Standard, requiring that, as of April 15, 2016, all direct connected and canopy connected Type A BSCs need to contain auxiliary airflow alarms for personnel safety. Accredited individuals and companies may no longer certify Type A cabinets unless airflow alarms are present. Therefore 14 of the Class II Type A BSCs at UTA that are connected to the building exhaust system were installed with additional alarms to be eligible for NSF field certifications.

Please Note: Any Class II Type A cabinet not connected to an exhaust system uses an air diffuser that also protects the exhaust filter. Make sure that the exhaust diffuser is not missing on top of the BSC. This filter is very fragile and easily damaged. Make sure the diffuser is installed correctly and not blocked. Keep the top of the cabinet clear.

Remember:

The main purpose of a BSC is to protect the user and the environment from exposure to biohazards while working with infectious agents/materials. In addition, Class II BSCs will protect the research material(s) from airborne contaminants with the aid of HEPA / ULPA supply filters.



Proper Use of Biosafety Cabinet

A biosafety cabinet (BSC) is a valuable supplement to good sterile technique and a containment device when working with biohazardous agents/materials. It is advisable not to store too many items inside the cabinet; this would hinder the cabinet from performing at its full capacity and would compromise the sterility of materials *inside the BSC*, leading to contamination of handled samples, and to personnel exposure to biohazardous materials. If a BSC is not operated correctly, it will not provide adequate protection. Below are some “Golden Rules” when working within a BSC to avoid contamination of samples and personnel exposure to the agents they are working with.



Follow these start-up procedures when preparing for work in a BSC:

Plan your experiment. Prepare a written checklist of the items that are needed for the experiment. Gather these materials before beginning the work and be sure to include personal protective equipment (PPE) and waste disposal containers.

Turn on the cabinet. If the cabinet is not running, turn on the blower switch. Before using the BSC, let it run at least five minutes to purge airborne contaminants from the work area.

Turn off ultraviolet (UV) light if in use and ensure that the sash is in the appropriate position. UV radiation is hazardous to your health. Newer cabinets have a safety circuit that allows the UV light to activate only if the sash is completely closed. Do not adjust this important setting! An alarm sounds and flashes when the sash is opened too high or closed too low. The alarm can be silenced for five minutes at a time when loading large equipment and cleaning. Never work in the cabinet when the sash is not at the correct height.

Check certification date of the BSC. NSF International Standard/American National Standard for Biosafety Cabinetry - NSF/ANSI 49 - 2018 Biosafety Cabinetry: Design, Construction, Performance, and Field Certification establishes performance criteria and provides the

minimum testing requirements that are accepted in the United States. Cabinets that meet the Standard and are certified by NSF bear an NSF mark. The operational integrity of a BSC must be validated before it is placed into service, after it has been repaired or relocated, and annually thereafter by qualified technicians (accredited field certifiers).



Check Magnehelic gauge. Magnehelic gauge measures the air pressure that air is pushed through the HEPA/ULPA filter. The magnehelic gauge should read close to the setting at the last certification (record that reading for easy reference). A reading that is too high or too low may indicate a problem with the airflows. Do not work in the cabinet if the magnehelic gauge indicates a problem. Inform Principal Investigator (PI) / Laboratory Supervisor!

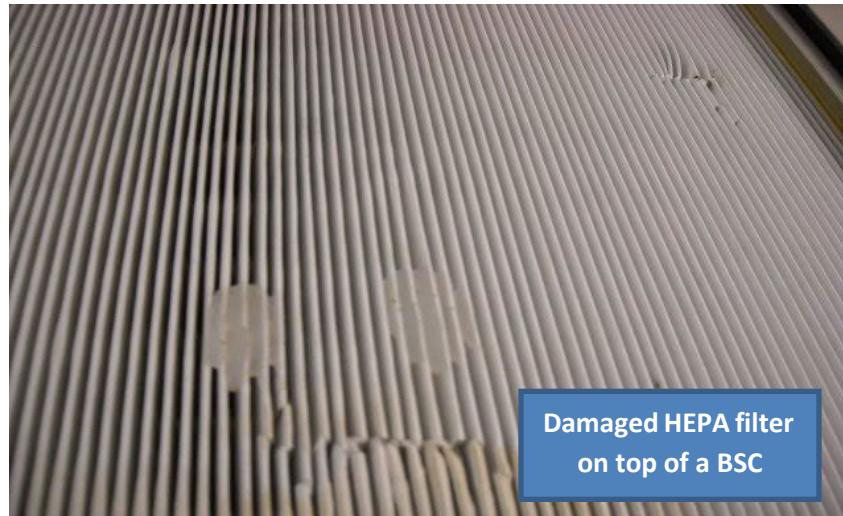
Confirm inward airflow. Hold a tissue at the middle of the edge of the viewing panel and ensure that it is drawn in.

Do not work in a BSC if a warning light or alarm is signaling.

PPE should be worn whenever using a BSC. Laboratory coat, gloves (pulled over the wrists of the lab coat, double gloving is recommended for some procedures), and safety glasses. Chemical splash goggles may be required for some procedures.

Disinfect the work surface, interior walls (not including the supply diffuser), and the interior surface of the window. Use a disinfectant appropriate for the work being conducted in the laboratory: 70% alcohol (ethanol/isopropanol), a 1:100 dilution of household bleach (i.e., 0.05% sodium hypochlorite), or other disinfectant as determined by the investigator to meet the requirements of the activity. Remove residual chlorine (could corrode the surfaces of BSC) by wiping the surfaces with 70% alcohol or sterile water. Check that the drain pan valve is closed before operation (if drain pan valve is present).

Reminder: Do not store supplies on top of the BSC. The HEPA/ULPA filter is located there, and it is easily damaged!



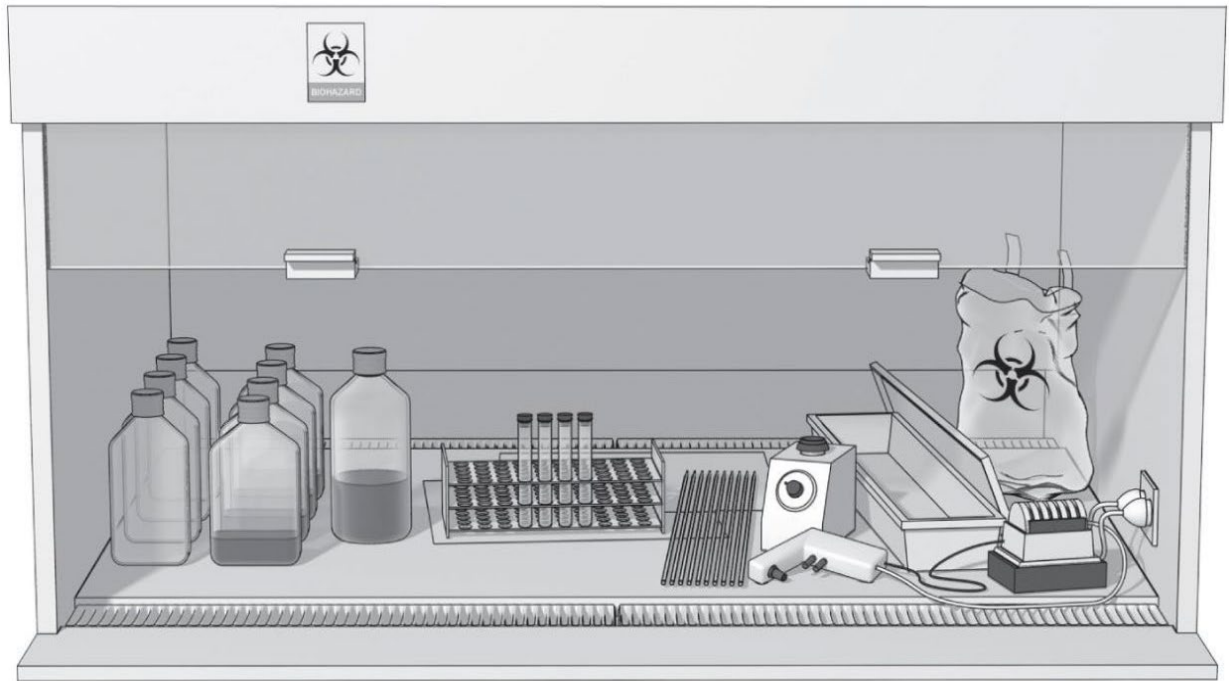
Follow these procedures to get ready to work in a BSC:

Prepare to begin work. Don protective clothing, safety glasses and gloves. Surface decontaminate all materials and containers with 70% alcohol before placing them into the cabinet. Deposit all items as far away from the sash as possible. Especially aerosol-generating equipment (e.g., vortex mixers, tabletop centrifuges) should be placed toward the rear of the cabinet. All operations should be performed on the work surface at least four (4) inches from the inside edge of the front grill. Try to keep the work surface as neat as possible. Work may be performed on disinfectant-soaked absorbent towels to capture splatters and splashes. Avoid overloading the work area, thereby compromising the efficacy of the BSC. Limit items inside the cabinet to only the most essential. Be sure not to obstruct the front and rear air grilles. When untreated laboratory air is blocked from entering the front grille, it can flow over the work surface contaminating the material worked with and posing a risk to personnel. Keep disinfectant and paper towels handy for spills. Wait five (5) minutes after all materials have been placed in the BSC before beginning work. This time is needed to purge airborne contaminants from the work area.



Use only the items essential to your task in the safety cabinet. Anything placed into the cabinet becomes contaminated—pens, pencils, clipboards, etc. If this occurs, always disinfect the item before taking it out.

Work from clean to dirty. Organize supplies so that work can be segregated from the clean side of the cabinet to the dirty side. Avoid moving dirty items over clean ones to prevent cross-contamination of the experiment. Avoid excessive movement of hands and arms through the front access opening during use. When you do enter or exit the cabinet, do so from straight on and allow the cabinet to stabilize before resuming work. Keep discarded, contaminated materials to the rear of the cabinet. A small biohazard collection bag needs to be located inside BSC. Do not discard materials in containers outside of the cabinet. Always use mechanical pipetting aids. Traffic around the BSC should be minimized and drafts from doors and air conditioning should be avoided. If there is a spill during use, surface decontaminate all objects in the cabinet and disinfect the working area of the cabinet while it is still in operation (do not turn the cabinet off). Aspirator bottles or suction flasks should be connected to an overflow collection flask containing appropriate disinfectant, and to an in-line HEPA or equivalent filter.



CLEAN → DIRTY

A typical layout for working "clean to dirty" within a BSC

Collect waste materials. Workers using infectious materials should collect their wastes inside of the cabinet. Pipettes should be decontaminated within the cabinet. Disinfect items in a shallow pan filled with disinfectant. Other biohazard waste (plastic pipette tips, gloves, paper towels, etc.) should also be bagged inside the cabinet to avoid spreading contamination. Repeatedly moving arms in and out of the cabinet to deposit waste in a container outside of the BSC will compromise the airflow and containment provided by the BSC.



Small biohazard collection bag and holder located inside BSC

Avoid using open flames (Bunsen burner) inside BSCs. Flames disrupt the airflow and contribute to the heat load inside the BSC. Flames can burn holes in HEPA/ULPA filters. Flammable gasses (gasses are not captured by HEPA/ULPA filters) may recirculate and build up to the Lower Explosive Level (LEL) and cause an explosion or fire. If a flame is necessary, use a burner with a pilot light and place it to the rear of the workspace.



Follow these procedures upon completion of the work in a BSC:

Surface-decontaminate objects / remove waste materials. Wipe down all objects with disinfectant before removing them from the BSC. All items within BSC, including equipment, should be surface-decontaminated and removed from the cabinet when work is completed, since residual culture media may provide an opportunity for microbial growth. Removing waste from the BSC that is untreated or not packaged in a leak-proof container can spread contamination. Be sure to seal any bags and cover open containers before removing them from the cabinet.

When removing arms from BSC, surface decontaminate gloves first and then move arms slowly out of the cabinet (in direction perpendicular to plane of work zone opening)!

Clean up. After the cabinet has been emptied, wipe down the interior cabinet surfaces (work surface, sides, back, and interior of the window) with a disinfectant. The cabinet should be allowed to run for at least three minutes with no activity so that the airborne contaminants will be purged from the work area. When the cabinet is shut off, ensure the sash is closed completely. Remove the work surface periodically and disinfect the area beneath it (including the catch pan).

Remove contaminated gloves and dispose of them as appropriate. Wash hands with soap and warm water.

Turn on the UV light if BSC is equipped with one. UV lights can accumulate dust and dirt that will block the germicidal effectiveness of the light. Bulbs can continue working for months or years after they stop providing the necessary wattage for disinfection purposes. The bulb should be wiped with alcohol weekly (while turned off) to remove dust. Additionally, UV light should be tested for efficacy to ensure that it is emitting a germicidal wavelength or bulb should be replaced annually. UV radiation should not take the place of chemical disinfection of the cabinet interior. Make sure the sash is closed completely before using UV light. The glass sash will protect you from UV radiation.

Common Errors to Avoid:

- Keep papers, paper towels, plastic backed absorbent toweling, vials, pipette wraps, or any other objects from being pulled into the back, front, or side slots of the BSC.
- Do not store equipment or supplies in the cabinet.
- Do not use the top of the cabinet for storage. The HEPA/ULPA filter could be damaged, and the airflow disrupted.
- Do not place items on the front or rear perforated grills. This reduces the airflow.
- Do not rest arms on the front grill. This reduces airflow.
- Make sure the cabinet is level. If the cabinet is not, the airflow can be affected.
- Never disengage the alarm, as it indicates improper airflow, thereby affecting performance and endangering the researcher or the experiment.
- Never completely close the window sash with the motor running. This may cause the motor to burn out.

Biohazardous Spills Inside Biosafety Cabinet

Follow these procedures when cleaning a small biohazardous spill inside a BSC:

- Decontamination steps should be initiated while the BSC continues to operate to prevent the escape of contaminants. Notify the PI before starting the spill cleanup.
- Notify co-workers of the spill.
- Wait at least five minutes to allow the BSC to contain aerosols.
- Wear lab coat, safety glasses and gloves during cleanup.
- Cover small spill with paper towels or other absorbent material. Slowly pour disinfectant solution (70% alcohol or other effective disinfectant) around the spill and allow to flow into the spill. Let the disinfectant work for 15 to 30 minutes.
- Dispose of the spill-disinfectant-soaked paper towels/absorbent in a biohazard bag.
- Wipe the spillage area with disinfectant-soaked paper towels.
- Spray or wipe the walls, work surface and any equipment in the cabinet with disinfectant-soaked paper towels and remove all equipment inside the BSC. Do not put your head inside of the BSC! Do not spray the HEPA filter!
- Discard contaminated disposable materials using appropriate biohazardous waste disposal procedure (Stericycle).
- Pick up any glass (or other sharps) with tongs or forceps and discard in a sharps container labeled with a biohazard sign.
- Place contaminated reusable items in autoclavable bags and store these closed bags in a secondary container before autoclaving is done.
- Expose non-autoclavable materials to disinfectant (20-minute contact time) before removal from the BSC.
- Remove all PPE and place protective clothing in an autoclavable bag for autoclaving.
- Wash your hands with soap and warm water.
- Run cabinet 10 minutes after cleanup before turning cabinet off.

If the spill in the BSC is quite substantial (spill flows past the work surface through the front or rear grilles), it may be necessary to decontaminate the cabinet's fans, filters, and airflow plenums. An outside company must do this. Call the EH&S Office at 817-272-2185 for assistance.

Biosafety Cabinet Decontamination Guidelines

Decontamination of a BSC is an important function in terms of both containment and sterility. Aseptic technique and daily decontamination will reduce/eliminate most contaminants. In addition, periodic and thorough decontamination routines (including disinfecting all removable parts and surfaces) is recommended by the manufacturer and is a good laboratory practice to reduce wear on the equipment and provide greater safety to the worker, samples that are worked with, and the environment.

Extreme caution should be observed when decontaminating BSCs. For example, use caution when wiping BSC surfaces to avoid injury from broken glass that may be present and sharp metal edges. When removing internal parts of the cabinet, it is best to work with a partner as some of the workbenches and grilles may be heavy and have sharp edges. Proper personal protective equipment (PPE) must be used.

The guidelines below address routine periodic decontamination of most BSCs used in BSL-1 and BSL-2 settings. Always refer to the manufacturer's operation and maintenance manual for instructions on periodic decontamination procedures. These guidelines are intended to be supplemental and are not a replacement for the manufacturer's recommendations.

The guidelines below do not refer to the decontamination procedure that would be performed by a third party (e.g., using formaldehyde gas) when BSC that has been used for work involving infectious materials must be decontaminated before high efficiency particulate air (HEPA) filters/ultra-low particulate air (ULPA) filters are changed, or when maintenance work is planned.

Follow these procedures when performing a periodic and thorough decontamination of a BSC:

Always wear appropriate PPE when performing decontamination procedures. At minimum, PPE includes a laboratory coat, gloves, and eye protection. Long pants and sturdy, closed-toe shoes that provide maximum foot protection needs to be used. Whenever possible, tuck lab coat cuffs inside gloves.

Prepare for the decontamination. Plan on at least one hour or more of uninterrupted time to complete a thorough disinfecting routine. Schedule this in advance with others in the lab to minimize down time. When performing a full decontamination, which includes removing internal parts (such as the grilles and workbench), it is best to set up an area adjacent to the BSC to place these items. Ensure that several layers of absorbent materials are placed on the floor to soak up the disinfectant being used. It is also a good idea to have a partner who can help move the heavier removable parts in and out of the BSC.

Determine the appropriate disinfectant based on the microbiological materials used in the BSC. Twenty to thirty minutes is generally considered an appropriate contact time for decontamination, but this varies with the disinfectant and the microbiological agent. Manufacturer's directions must always be followed. The use of chlorine bleach or halogen chemicals may damage the stainless-steel surfaces.

For example, when using a 1% bleach solution, follow with a sterile distilled water rinse to remove any residue or with 70% alcohol (or similar non-corrosive antimicrobial agent).

Decontamination procedure. Decontamination of BSC surfaces can be done using a spray bottle with a mist setting to liberally apply disinfectant. Using an up and down, left to right sweeping motion ensures all surface areas are completely covered and no area remains dry. Avoid spraying the ultraviolet (UV) light bulb and HEPA/ULPA filter. Always wait for the appropriate contact time to elapse, then wipe to remove excess liquid. If bleach was used as a disinfectant, follow with either sterile distilled water and/or 70% ethanol to remove any residue. A squeegee type tool or a duster with disposable pads (such as a clean room duster) can also be used to wipe the internal surfaces, rather than paper towels (which can cause lint). This is also more sustainable. A less expensive and effective alternative is a sweeper floor mop (e.g., Swiffer) with a shortened handle. A disposable dry cloth can be soaked with disinfectant and then used to easily apply disinfectant evenly to all surfaces. Remove and discard the cloth into the biohazard waste after use and repeat the procedure with a fresh cloth soaked with sterile distilled water or 70% alcohol if bleach solution was used for decontamination.



- Turn on the BSC in normal operational mode with internal blower and light on.
- Check the pressure on the magnehelic gauge (the pressure should be the same after cleaning and if not, could indicate the HEPA/ULPA filter was disrupted and the BSC may require recertification).
- Open the sash for normal operation and allow for the normal purge time of at least five minutes before starting the decontamination of the BSC.
- Spray disinfectant on a biohazard waste bag and place it in the BSC to collect the used absorbent materials.
- Remove and place any small removable parts on the workbench surface.
- Working from left to right and top to bottom, spray disinfectant on all internal surfaces: the left wall, back wall, and right wall. Next, spray the workbench and the entire inside of the glass sash. Allow the disinfectant to soak while conducting the next steps. Alternatively, use disposable dry cloths soaked with disinfectant as described above.
- Carefully loosen any thumbscrews (if applicable) to release the removable workbench surface.
- To ensure thorough cleaning of the workbench area, all the parts need to be removed and cleaned outside of the BSC. Using a partner, remove the front perforated grille and removable workbench surface. Prop up against a sturdy surface adjacent to the BSC (e.g., lab bench) by positioning perpendicular on the floor on top of several layers of absorbent materials (e.g., lab bench protectors, soakers).
- Clean the front perforated grille and removable workbench surfaces by spraying disinfectant onto the outward facing surfaces. Allow for appropriate contact time to elapse before wiping. Turn the pieces over and repeat the cleaning process. Alternatively, use disposable dry cloths soaked with disinfectant as described above.
- Once the grille and workbench have been thoroughly cleaned outside of the BSC, remove gloves, and wash your hands with soap and water and put on a new pair of gloves before re-entering the BSC to complete the disinfection process under the workbench surface now that it has been removed.
- Inspect under the workbench surface. Carefully remove any debris such as paper towels, glass beads, stray sharps, tubes, Pasteur pipettes, ampoules, or broken glass. Use tongs or forceps for glass!
- Disinfect the under-workbench surfaces including tray supports and the plenum drain area. Rinse by spraying sterile distilled water (if required) and follow with spraying 70% ethanol. Wipe dry.
- Using a partner, carefully replace and reposition the grille and removable workbench surface back into the BSC.
- Tighten any thumbscrews to secure the workbench surface in place (if applicable).
- Disinfect the grille and workbench surface.
- Working from left to right and top to bottom, spray with sterile distilled water (if rinse is required) or spray 70% alcohol on all internal surfaces: the left wall, back wall, and right wall, then the workbench and the entire inside of the glass sash. Wipe dry.

- Transfer all waste materials into the biohazard bag, close the bag inside of the cabinet, and wipe with disinfectant prior to removal.
- Repeat the check of the pressure reading on the magnehelic gauge (the pressure should be the same after cleaning and if not, could indicate the HEPA filter was disrupted and the BSC may require recertification).
- Let the BSC blower operate 10 minutes after decontamination before turning it off.
- Remove all PPE and place protective clothing in an autoclavable bag for autoclaving.
- Wash your hands with soap and warm water.



Special Decontamination Considerations:

Periodic and thorough disinfection also provides the opportunity to inspect and replace vacuum system parts and tubing, and to disinfect small equipment used in the BSC.

The chairs should also be regularly disinfected, paying particular attention to the areas of the chairs where gloved hands may have touched.

Revised 05-08-2023