

# **THE UW-MADISON MANUAL FOR BIOSAFETY CABINETS, ANIMAL TRANSFER STATIONS, AND CLEAN AIR DEVICES**

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## **LABORATORY CONTAINMENT EQUIPMENT**

Containment equipment such as chemical fume hoods, biological safety cabinets (BSC), animal transfer stations (ATS), and clean air devices (CAD) are used in research to provide protection.

There are three basic types of protection:

- **Personal protection** is the protection of the people working in the laboratory.
- **Product protection** is the protection of the product or experiment.
- **Environmental protection** is the protection of the environment outside of the laboratory.

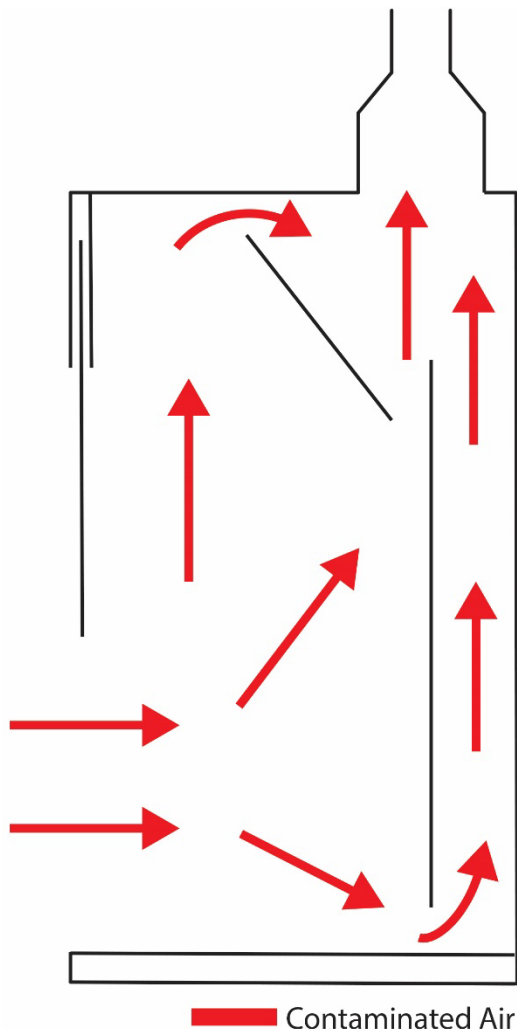
Directional air flow, air curtains, and HEPA filtration are used to provide protection. Directional air flow into BSCs and fume hoods and an air curtain at the sash contain contaminated air inside the units affording personal protection. Whereas HEPA filtration of laminar flowing supply air offers product protection and HEPA filtration of exhausted air offers environmental protection.

Determining which type of equipment to use depends on the materials (e.g. chemicals, biologicals) handled and the type of protection (i.e. personal, product, environment) needed. Be sure you understand the differences between chemical fume hoods, biological safety cabinets, animal transfer stations, and clean air devices.

### **Chemical Fume Hoods**

Characteristics of chemical fume hoods:

- Provide only personal protection.
- Exhausts air to the outside of the building.
- Do not offer product or environment protection, as there is no filtration of intake and exhaust air. Sometimes air cleaning treatment or HEPA filters are added to provide a layer of protection.
- Directly draws air from the laboratory over the product in the hood (Figure 1).



Chemical fume hoods are used for work with chemical hazards and is not considered to be biocontainment equipment. The use of a fume hood with biological materials instead of a BSC must be approved by the Office of Biological Safety and/or the Institutional Biosafety Committee.

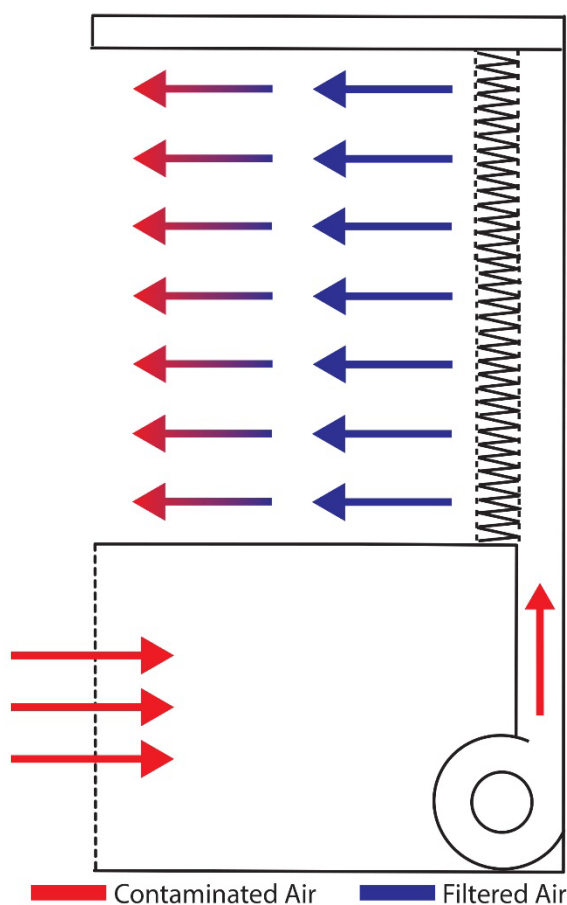
**Figure 1. Fume hood airflow pattern.** Dirty lab air is directed across the work surface away from the user to provide personal protection. Fume hoods are connected to the building exhaust system to exhaust air to the outside.

### Clean Air Devices (CAD)

Characteristics of CADs:

- Provides only product protection.
- Creates a laminar (i.e. unidirectional) airflow generated through a HEPA filter to provide product protection.
- Discharges air across the work surface, towards the user and into the workroom.

Clean air devices may also be known as laminar flow hood, horizontal flow hood, or clean benches. Some may look similar to a BSC but do not provide the same level of protection. In fact, the risk of illness (e.g. lab acquired infection, chemical exposure) is greater if potentially hazardous biological or chemical materials are handled inside a clean air device due to the air directed across the work surface towards the user (Figure 2). **Thus, CADs must never be used for work with these materials in order to prevent exposure to or release of biological materials.** CADs may be used for applications where the product is not hazardous but must be kept contaminant free such as for the preparation of nonhazardous mixtures and media, or the particulate-free assembly of sterile equipment and electronic devices. Animal or plant pathogens could be handled in CADs if there is no concern for spread into the animal colony or the environment. With that said, the use of animal or plant pathogens in a CAD must be approved by OBS or the IBC.



**Figure 2. CADs airflow pattern.** HEPA-filtered air is directed across the work surface towards the user to provide clean air for the product but exposes the user to materials used inside the CAD.

### Animal Transfer Stations (ATS)

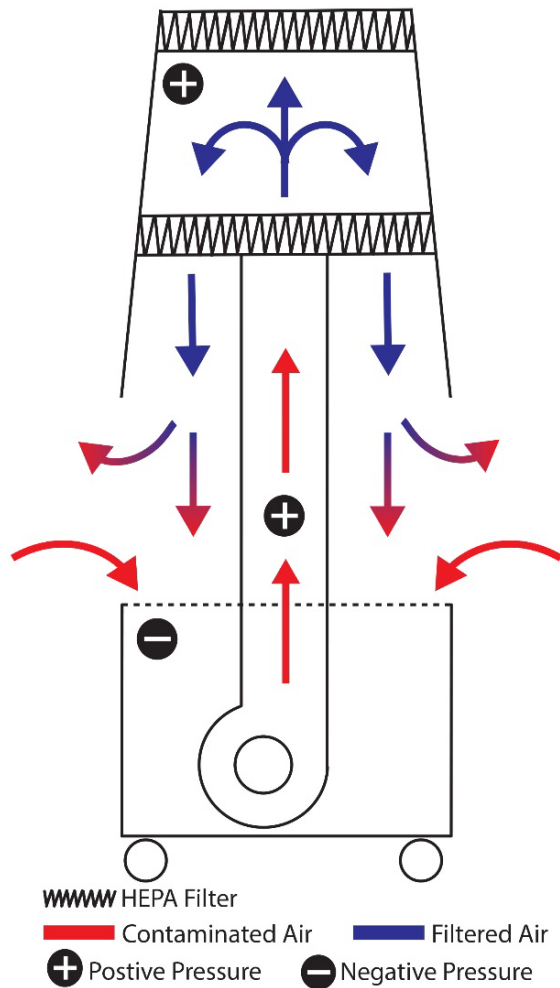
Characteristics of ATSs:

- Creates a unidirectional airflow generated through a HEPA filter to provide clean air to protect animals

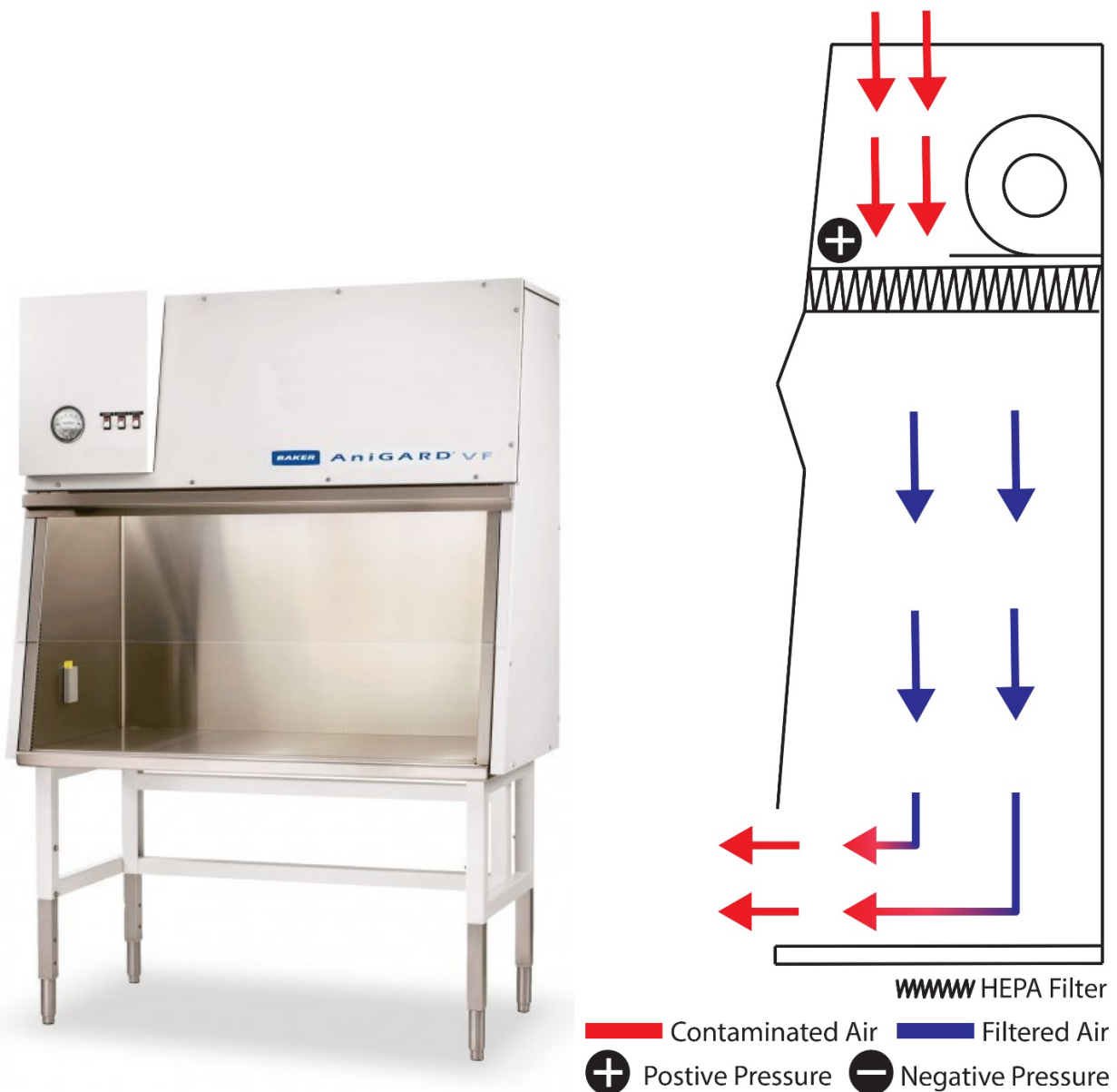
- Some models use directional airflow to reduce personal exposures to animal allergens though they do not provide sufficient personal protection for work with hazardous biologicals

Animal transfer stations are similar to clean air devices and are designed to protect animals from spreading of disease. Although some models of ATSs (e.g. three-sided ATS) provide some personal protection by reducing exposures to animal allergens and soiled bedding during cage changes, they do not provide sufficient protection for work with hazardous materials (Figure 3). A different type of ATS (e.g. vertical flow ATS) does not provide any personal protection (Figure 4). **In order to protect users and BSC certifiers, ATSs must never be used for work with potentially hazardous biological or chemical materials nor for cage changes at ABSL2 or higher regardless of PPE worn.** The intended use of animal transfer stations is for animal handling and cage changes at ABSL1 and must not be used for handling of any biological materials.





**Figure 3. Three-sided ATS airflow pattern.** This three-side ATS directs HEPA-filtered air at the work surface to help protect animals whereas the directional airflow and HEPA-filtered exhausted air provides some level of protection to users. However, gaps below the sashes do not provide sufficient protection to users to allow use with hazardous biologicals



## **Biological Safety Cabinet (BSC)**

Characteristics of BSCs:

- Designed to contain biological hazards and to allow products to be handled in a clean environment.
- Have an inward airflow for personal protection.
- Create a unidirectional airflow generated through a HEPA filter to provide product protection (except Class I).
- HEPA filter exhaust air for environmental protection.

BSCs are the primary means of containment developed for working safely with infectious microorganisms. They can be used with microorganisms, cell cultures, and pharmaceuticals. BSCs are designed to provide personal, environmental, and product protection when certified and used correctly in conjunction with good microbiological techniques. An excellent reference is *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* "Appendix A - Primary Containment for Biohazards: Selection, Installation, and Use of Biological Safety Cabinets", published by U.S. Department of Health and Human Services, Public Health Services, Centers for Disease Control and Prevention (CDC) and National Institutes of Health (NIH).

## **CLASSIFICATION OF BSCs**

Three classes of biological safety cabinets, designated as Class I, II, and III, have been developed to meet varying research and clinical needs. Table 1 summarizes the major characteristics of these types of BSCs in addition to chemical fume hood, CADs, and ATs. Unless the specific class of BSC is indicated, the use of the word BSC in this manual refers to Class II BSCs.

### **Class I BSC**

Characteristics of Class I BSCs:

- Provides personal and environmental protection but not product protection.
- Does not provide product protection because it lacks HEPA-filtered supply air.
- May exhaust HEPA-filtered air into the room or can exhaust to outside if connected to the building exhaust system.

### **Class II BSC**

Characteristics of Class II BSCs:

- Provides personal, product, and environmental protection.
- Five types of Class II BSCs based on air flows achieved and type of exhaust (see below for more information)
  - Type A1
  - Type A2
  - Type B1
  - Type B2
  - Type C1

- Includes a third type of ATS which is classified as a Class II Type A1BSC and provides the same level of protection while offering a larger sash opening and manufactured-installed wheels. To avoid confusion, this type of unit is called a BSC on the UW-Madison campus.

### **Class III BSC**

Characteristics of Class III BSCs:

- Provides maximum level of personal, product, and environmental protection that can be used at BSL1-BSL4.
- Gas-tight containment enclosure with gloved ports for handling materials in the unit.
- Dual HEPA filter exhaust (i.e. air is passed through two HEPA filters).
- May also be called an isolator or glove box but should not be confused with anaerobic chambers.

Class II BSCs are the most common class of BSCs used at UW-Madison. Within this class, there are five types in which Class II Type A2 is the most common found on this campus.

### **Class II Type A1 BSC**

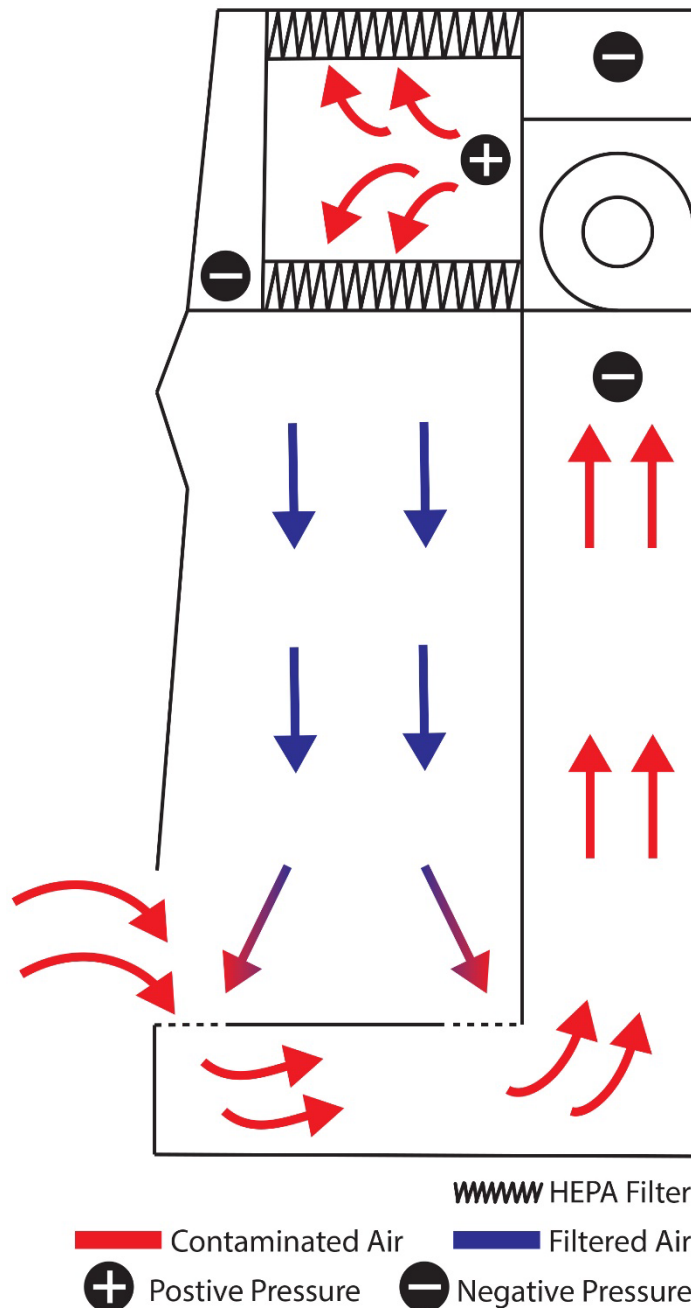
Characteristics of Class II Type A1 BSCs:

- Operates with an inward airflow rate of 75 feet/min.
- Recirculates air in which 70% is HEPA-filtered and recirculated back to the work surface while 30% is HEPA-filtered and exhausted.
- May exhaust HEPA-filtered air into the room or can exhaust to outside if connected to the building exhaust system through a canopy connection

### **Class II Type A2 BSC**

Characteristics of Class II Type A2 BSCs (Figure 5):

- Operates with an inward airflow rate of 100 feet/min.
- Recirculates air in which 70% is HEPA-filtered and recirculated back to the work surface while 30% is HEPA-filtered and exhausted.
- May exhaust HEPA-filtered air into the room or can exhaust to outside if connected to the building exhaust system through a canopy connection.



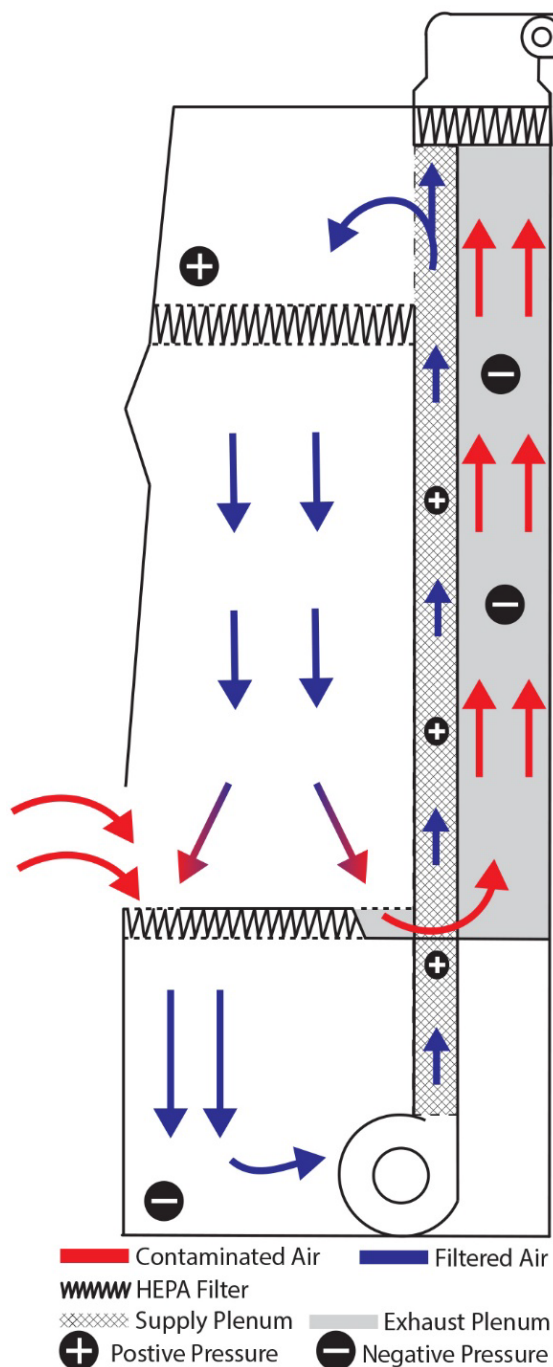
**Figure 5. Class II Type A2 BSC Airflow Pattern.** Dirty room air enters through the front grille of the BSC and is HEPA-filtered prior to entering the work surface. Air is recirculated within the BSC with a smaller percentage exhausted. Volatile chemicals can be concentrated due to recirculation and should not be used in this type of BSC.

### Class II Type B1 BSC

Characteristics of Class II Type B1 BSCs (Figure 6):

- Operates with an inward airflow rate of 100 feet/min.
- Has a zoned work area in which the front portion recirculates 30% of the HEPA-filtered air and exhausts the balance while the back portion exhausts 100% of the HEPA-filtered air.

- Are hard ducted to the building exhaust system and exhausted to the outside.



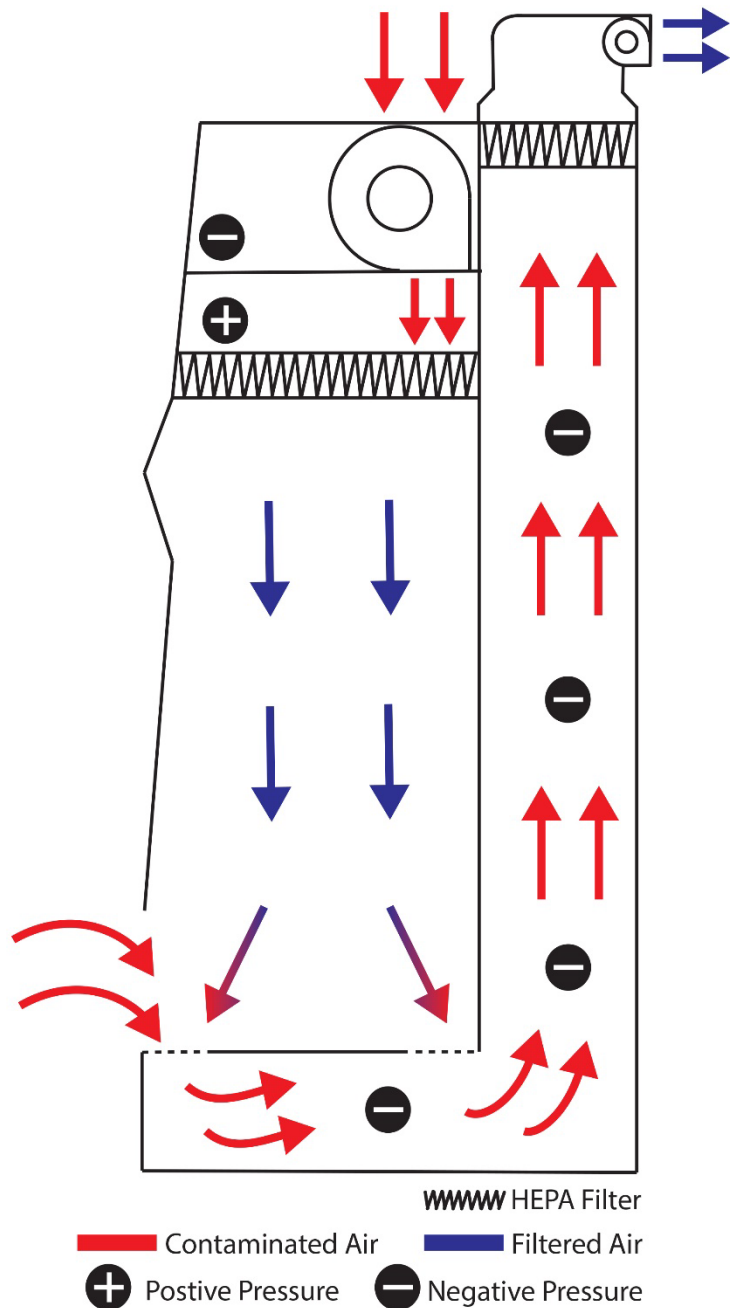
**Figure 6. Class II Type B1 BSC Airflow Pattern.** Dirty room air enters through the front grille of the BSC and is HEPA-filtered prior to entering the work surface. Air from the front portion of the work surface is recirculated within the BSC while air towards the back portion of the work surface is 100% exhausted through the building exhaust system. Volatile chemicals should be handled towards the back of the BSC otherwise they can be concentrated to levels that pose an explosive and/or health hazard.

### Class II Type B2 BSC

Characteristics of Class II Type B2 BSCs (Figure 7):

- Operates with an inward airflow rate of 100 feet/min.

- Air is not recirculated (i.e. 100% of air is exhausted).
- Are hard ducted to the building exhaust system and exhausted to the outside.



**Figure 7. Class II Type B2 BSC Airflow Pattern.** Dirty room air enters through the front grille of the BSC and is HEPA-filtered prior to entering the work surface. There is no recirculation of air, allowing for the safe use of most chemicals in the BSC. All air from the work surface is exhausted through the building exhaust system.

### Class II Type C1 BSC

Characteristics of Class II Type C1 BSCs:

- Operates with an inward airflow rate of 100 feet/min.

- Has a zoned work area in which air in one zone may be recirculated while air in the other zone is not.
- May exhaust HEPA-filtered air into the room or can exhaust to outside if connected to the building exhaust system through a canopy connection.

**Table 1. Containment Equipment**

<b>Device</b>	<b>Protection</b>	<b>Airflow Direction</b>	<b>Application/Airflow Pattern</b>	<b>Use of Volatile Toxic Chemicals and Radionuclides</b>
Chemical fume hood	Personnel	Inward	A completely exhausted, unfiltered device used for work with chemical hazards, minimizing exposure to personnel.	Acceptable
Clean air device, Clean bench	Product	Outward	A laminar flow clean air device provides HEPA filtered supply to the work surface and a particulate-free work area. For any application where the product is not hazardous but must be kept contaminant free (e.g. preparation of nonhazardous intravenous mixtures and media, particulate-free assembly of sterile equipment and electronic devices, polymerase chain reaction (PCR)).	Not Acceptable
Animal transfer station	Product	Inward or outward depending on model	A HEPA-filtered device used to transfer animals from dirty to clean cage, minimizing exposure to animal and personnel.	Not Acceptable
Bedding dump station	Personnel and environment	Inward	A HEPA-filtered device used to capture airborne particulates when disposing of waste bedding from animals, minimizing exposure to personnel.	Not Acceptable
BSC Class I	Personnel and environment	Inward	Similar to a fume hood in that it does not provide product protection. Exhaust air is HEPA filtered.	Acceptable if connected to exhaust <sup>1, 2</sup>

BSC Class II– A1	Product, personnel, and environment	Inward	A laminar flow device that recirculates 70% of its airflow to the work surface through a HEPA filter and exhausts the 30% balance through a HEPA filter back into the room or to the outside through a thimble connection via building exhaust system. Plenums in units built prior to 2008 are under positive pressure.	Minute amounts only if thimble connected to exhaust <sup>1</sup>
BSC Class II– A2	Product, personnel, and environment	Inward	A laminar flow device that recirculates 70% of its airflow to the work surface through a HEPA filter and exhausts the 30% balance through a HEPA filter back into the room or to the outside through a thimble connection via building exhaust system. Plenums are under negative pressure.	Minute amounts only if thimble connected to exhaust <sup>1</sup>
BSC Class II– B1	Product, personnel, and environment	Inward	A laminar flow device that recirculates 30-40% of its airflow to the work surface through a HEPA filter and exhausts the 60-70% balance through a HEPA filter to the outside via building exhaust system. Exhaust connection must be hard ducted to the outside.	Limited amounts <sup>1</sup>
BSC Class II– B2	Product, personnel, and environment	Inward	A laminar flow device that has a dedicated HEPA filtered supply to the work surface and a dedicated HEPA filtered exhaust to the outside via building exhaust system. No recirculated supply, and exhaust connection must be hard ducted to the outside.	Acceptable <sup>2</sup>
BSC Class III, Isolator , Glove box	Maximum product, personnel, and environment	Inward	A laminar flow device with dedicated HEPA filtered supply to the work surface and dual dedicated HEPA filtered exhausted to the outside via building exhaust system. No recirculated supply, and exhaust connection must be hard ducted to the outside. (e.g., pharmaceutical quality control testing, super-clean manufacturing without creating clean room, pharmaceutical manufacturing of potent compounds, BL4 agents).	Limited amounts <sup>1</sup>

<sup>1</sup> In no circumstances should the chemical concentration approach the lower explosion limits of a compound.

<sup>2</sup> Large amounts of volatile chemicals should be evaluated to verify they will not damage the exhaust HEPA filter.

*Sources:* Adapted from NSF Standard 49 and *Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets*, Current Edition. U.S. Department of Health and Human Services, Public Health Services, Centers for Disease Control and Prevention and National Institutes of Health

## COMPONENTS OF BSCs

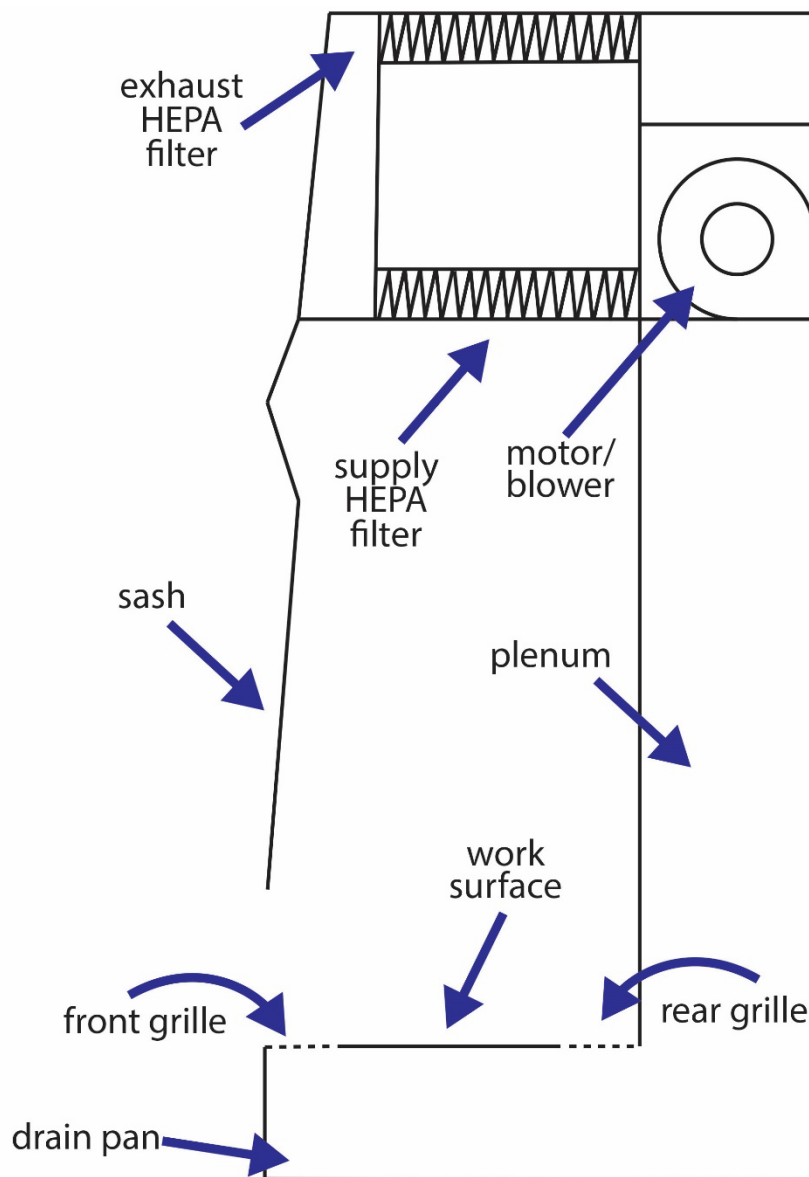
A diagram of various components of BSCs can be found in figure 8. BSCs consist of the following components:

- **Sash** or view screen aids in containment when used at the proper height
- **Front/Rear grilles** help to establish the protective airflow pattern.
- **Plenum** is the enclosed space along the sides, backs, and other areas that is used for airflow
- **Motor/blower** blows air through the BSC
- **Supply HEPA filter** to remove airborne contaminants from air supplied to the work surface
- **Exhaust HEPA filter** to remove airborne contaminants from air as it is exhausted out of the BSC
- **Alarms** alert users when BSCs are not used properly (e.g. sash height) or when containment is compromised due to disruption to the BSC's airflow (e.g. power failure or loss of building exhaust)
- **Pressure gauge** displays the pressure differential within a BSC to help indicate how the BSC is functioning. On BSCs manufactured by Baker, the gauge is located in the front on the upper left side.
- **Work surface** is the area used inside the BSC
- **Drain pan** is located beneath the work surface to capture liquid that may have spilled below the work surface.
- **Drain valve** is used to drain the drain pan. The drain valve is typically located beneath a lower, front corner of the cabinet.
- **Visible lights** illuminate the work surface for user

Optional Components:

- **UV lights** have germicidal affects if used properly. However, 60% of its effectiveness is lost after 40 hours of use.

- **Flexair canopy** connects a Class II Type A BSC to a building's exhaust system and maintains correct air flow in the event of fluctuations in the building exhaust system
- **Canopy connections** allow for Class II Type B BSCs to be directly connected to the building exhaust system to exhaust volatile chemicals or gases
- **Air/CO<sub>2</sub>/vacuum connections with flexible lines** allow for these services to be plumbed to the BSC



**Figure 8. Components of a BSC.**

## HEPA Filtration

Air filtration works by forcing air through a medium that traps particles. HEPA filters trap 99.97 percent of particles 0.3 microns in diameter. It is the least effective at filtering particles of this size whereas smaller or larger particles are more efficiently trapped due to diffusion, interception, or impaction.

### **Exhaust Options for BSCs**

Whether the air from a BSC should be exhausted into a room or through the building exhaust system depends on the materials used inside the BSC. HEPA filters capture particulates such as microorganisms but do not capture volatile chemicals or gases. When hazardous materials are used that cannot be captured by the HEPA filter (e.g. volatile chemicals or gases), the BSC is connected to the building exhaust system. A chemical risk assessment is performed by UW-Madison Chemical Safety to determine the type of BSC and the exhaust configuration needed for work with volatile chemicals and gases inside BSCs. See the section Use of Chemicals in CADs, ATSSs, and BSCs for more information regarding chemical usage inside BSCs.

### **Alarms**

Alarms warn users when there are conditions that could compromise protection.

An alarm will sound under the following conditions:

- Improper sash height
- Power failure
- Loss of building exhaust (only applies to BSCs connected to the building exhaust system)

For more information regarding alarms including what to do in the event of an alarm, please go to section called Procedures in the Event of an Alarm.

### **Ready Safe Mode**

Ready Safe Mode is a feature in newer BSCs that allows for users to keep the sash closed while safely leaving the motor/blower operating. This provides a cleaner environment inside the BSC while not in use. Currently, the following models of BSCs manufactured by Baker have this feature: SG403A-HE, SG603A-HE, SG404, SG604, BCG401, BCG601. BSCs without this feature should be turned off whenever the sash is closed. If you are unsure if your BSC has Ready Safe mode, please contact the BSC Certification Program (608-262-1809; [bscservices@fpm.wisc.edu](mailto:bscservices@fpm.wisc.edu)).

### **Armrest**

Even though the armrest appears to only serve an ergonomic purpose, BSCs are engineered and certified with the armrests in place. Removing the armrest can impact airflows and containment; therefore, they should never be removed. If the foam needs to be reattached or replaced, please contact the BSC Certification Program (608-262-1809; [bscservices@fpm.wisc.edu](mailto:bscservices@fpm.wisc.edu)).

## USE OF BIOLOGICALS IN BSCs, ATSSs, AND CADs

The type of containment equipment to use is decided based on the type of protection needed. It is important to use the correct equipment to ensure the protection of people, research samples, animals, plants, and our environment.

The use of biocontainment equipment is not required for biological materials that are not infectious or hazardous to humans, animals, or the environment. However, for procedures where the biological material is not hazardous but must be kept contaminant-free, the use of CADs or BSCs may be desired.

CADs only provide product protection and shall not be used with potentially hazardous biologicals in order to protect users and certifiers. Instead, a BSC should be used and may be required based on the laboratory's approved biosafety protocol.

Animal transfer stations are used for colony disease management and to reduce animal allergen exposures to users during cage changes. They are not biological safety cabinets and shall never be used for work with potentially hazardous biologicals in order to protect users and certifiers. In addition, ATSSs shall never be used for cage changes at ABSL2 or higher due to an increased risk of exposure to certifiers and the difficulty or inability to gas decontaminate for repairs or disposal.

Table 2 provides a summary of the type of materials that can be used and activities that can be performed in CADs, ATSSs, and BSCs. The biosafety protocol approved by OBS or the IBC dictates whether or not the use of a BSC is required.

**Table 2. Summary of Material Usage and Activities Performed in CADs, ATSSs, and BSCs**

	CAD	ATS	BSC
Non-hazardous biological <sup>1</sup>	Yes	No	Yes
RG1 Opportunistic Pathogen <sup>2</sup>	No	No	Yes
RG2 Human pathogen	No	No	Yes
RG3 Human pathogen	No	No	Yes
Animal pathogen	Depends, contact OBS	No	Yes
Plant pathogen	Yes	No	Yes
ABSL1 cage changes	Requires OBS approval	Yes	Yes

ABSL2-ABSL3 cage changes	No	No	Yes
Animal Inoculations	Depends, contact OBS	No	Yes
Chemical Usage	Depends, contact Chemical Safety	No	Depends, contact Chemical Safety

<sup>1</sup>Non-hazardous biologicals are those that are not pathogenic to humans, animals, or plants.

<sup>2</sup>RG1 microorganisms that are opportunistic pathogens as defined by the IBC Policy 19, are handled at BSL2 and the use of a BSC may be required by OBS or the IBC.

## USE OF CHEMICALS IN BSCs, ATSSs, AND CADs

The ideal location for the use of hazardous chemicals is inside a chemical fume hood. If chemicals are to be used in other containment devices, there are some factors to consider:

- The specific physical and health properties of each chemical used
- How the chemical is used
- The quantity used
- The type of containment device used
- The airflow of the device (recirculating, connected to exhaust, etc.)

Generally speaking, no hazardous chemicals should be used in devices in which air flows towards the user (e.g. ATSSs, CADs). These devices are not designed for user protection and may expose workers to hazardous materials if such materials are used inside of the enclosure. If this use is desired, a chemical risk assessment needs to be performed by UW-Madison Chemical Safety.

BSCs offer the potential for some chemical use, depending on the specific type and properties of the BSC. Every process is unique, and any chemical use inside of a BSC should include a risk assessment by the laboratory, focusing on the factors identified above, and consulting with Chemical Safety when necessary. In particular, Chemical Safety should be consulted if the process involves unusual hazardous chemicals, not typical of biological laboratories, to evaluate if BSC use is appropriate. Best practice for working with any chemical in a BSC is to use the smallest amount of material as practical, and keep the container closed whenever not actively using. This will reduce the likelihood and severity of any potential spill. The use of reactive chemicals, such as explosive, pyrophoric, or water reactive chemicals is not appropriate in these devices.

HEPA filters are quite efficient at removing particulates. For this reason, the use of non-volatile, solid chemicals is generally acceptable in all BSC types. If the desired protection is solely from powders, other protection devices such as a powder weighing station may be appropriate, as large amounts of powders can prematurely block airflow through the HEPA filter. Non-volatile liquids, including aqueous solutions, can typically be handled safely inside of BSCs as well.

HEPA filters do not block the movement of small molecules, such as gases and vapors. Hazardous gases shall not be used in a BSC without consulting with Chemical Safety. Furthermore, the use of volatile chemicals should also be evaluated by Chemical Safety. This is for two main reasons. First, the inside of a BSC often contains potential ignition sources, such as electrical outlets, so care must be taken to avoid explosive concentrations of flammables. Second, many volatile chemicals have the ability to degrade HEPA filters. This will reduce the BSCs effectiveness at protecting users, samples, and the environment.

Class II Type A1 and A2 units that exhaust air into the room shall not be used with hazardous chemicals without consulting with Chemical Safety. OSHA recommends the use of fume hoods for volatile chemicals with a threshold limit value (TLV) of 50 ppm or lower. A fully ducted BSC (Class II Type B) is also appropriate when using smaller volumes of chemicals, determined in consultation with Chemical Safety.

Recirculating BSCs which are connected to building exhaust (Class II Type B1, or canopy connected Class II Type A1 or A2) can allow for some chemical use, but there is still a risk of concentrating chemical vapors inside of the BSC. The Class II Type B1 has a greater ratio of exhausted air than the A1 or A2, increasing the potential volume of chemicals used. Amounts greater than minute need to be evaluated by Chemical Safety.

The best time to evaluate which type of BSC is most appropriate is before purchase and installation, and Chemical Safety is available to assist in that process.

## **USE OF RADIOISOTOPES IN BSCs, ATSSs, AND CADs**

Generally, the use of small amounts of non-volatile radionuclides can be done safely in a BSC. Common procedures include tritiated thymidine incorporation, cytotoxicity assays using Cr-51, cell labelling with S-35 methionine, and incorporation of P-32 orthophosphate or C-14 labelled compounds.

The use of plastic backed absorbent bench paper to protect the work surface during radioactive experiments should be considered. This makes cleanup easy and reduces chance for contamination of the work surface.

In order to protect personnel, researchers must follow certain procedures:

- The room where the BSC is located must be attached to an active radioactive materials permit
- The nuclide(s) used in the BSC must be listed on the permit

- The BSC must be clearly posted with a Caution – Radioactive Materials sticker
- After each use of radioactive materials in a posted BSC, the BSC must be surveyed with an appropriate survey meter, or by wipe test in the case of H-3 or C-14 use.
  - The survey must be documented
  - Contamination greater than three times background found using a meter requires a follow-up wipe survey
  - Wipe survey decontamination limits are listed below. Decontaminate areas where contamination greater than or equal to
    - 2000 DPM – Radiation Use Area(s)
    - 200 DPM - All other areas
- The BSC must be included on your 30 day survey
  - Use contamination limits listed above
- The Office of Radiation Safety (ORS) must decommission the BSC
  - Before any certifications, repairs, maintenance, or disposal of a posted BSC
  - If a BSC is no longer to be used with radioactive materials
  - If a BSC is to be relocated

Please give ORS at least a one-week notice for decommissioning. It takes time to take the samples and analyze them. If ORS finds contamination, the lab must decontaminate the BSC and ORS will resurvey.

If you have any questions regarding the safe use of radioactive materials in your BSC, do not hesitate to contact the health physicist assigned to your building, or e-mail the Office of Radiation Safety (ORS) at [radiationsafety@wisc.edu](mailto:radiationsafety@wisc.edu). We are here to help.

## OVERVIEW FOR PROPER USE OF A BSC

### Loading Materials/Equipment and BSC Startup

- ✓ Always keep doors closed to laboratory when working with any biohazardous materials.
- ✓ Turn on blower at least 10 minutes before use and make sure drain valve is closed.
- ✓ Check pressure gauge(s) to ensure proper operating conditions are within range of those indicated on the annual certification label on the BSC.
- ✓ Check grilles for obstructions.
- ✓ Disinfect all interior work surfaces with a disinfectant appropriate for the agent in use in accordance with lab's approved biosafety protocol, if applicable. Ensure that manufacturer recommended contact time is observed. Follow up with either water or ethanol to prevent the disinfectant from corroding the metal working surfaces.
- ✓ Disinfect the exterior of all containers prior to placing them in the cabinet.
- ✓ Load only items needed for the procedure.
- ✓ Arrange materials so that movement within the cabinet is minimized; flow of procedure is from clean to dirty. Never place non-sterile items upstream of sterile

items. Check that rear and front grilles are unobstructed. Never hang articles from the interior walls or interior ceiling grid.

- ✓ Once the cabinet is loaded, adjust the sash to the proper position and wait 4 minutes before commencing procedures. Never use the sash above the mark specified by the certification agency (common opening is 8-inches and up to 12" for animal facilities)
- ✓ Restrict traffic in the vicinity of the BSC.

### **Recommended Work Techniques**

- ✓ Wash hands thoroughly with soap and water before and after procedures.
- ✓ Wear new disposable gloves and clean lab coat/gown and eye protection; use aseptic technique.
- ✓ Avoid blocking front grille. Work only on or over a solid surface and adjust the chair so your armpits are at the level of the lower window edge.
- ✓ Avoid rapid movement during procedures, particularly within the BSC, but also in the vicinity of the BSC.
- ✓ Move hands and arms straight into and out of the work area; never sweep or rotate hand/arm out of work area during procedure. Move laterally in work area.
- ✓ Do not use heat generating devices inside a BSC as the flame causes air turbulence and the use of gases for Bunsen burners could cause a fire or explosion. Use alternative equipment, such as disposable loops.
- ✓ Place contaminated items such as pipettes in a waste receptacle located within the BSC to reduce the frequency of crossing the air curtain.

### **Final Purging and Wipe-Down**

- ✓ After completing work, run the BSC blower for at least 10 minutes before unloading materials from the cabinet.
- ✓ Disinfect the exterior of all containers before removing them from the work zone.
- ✓ Decontaminate interior work surfaces of the BSC with an appropriate disinfectant effective against the agent used in accordance with lab's approved biosafety protocol, if applicable. Do not place your head inside the BSC, instead use cleaning tools with longer handles (e.g. Swiffer with extendable handle) for hard to reach surfaces. Follow up with either water or ethanol to prevent the disinfectant from corroding the metal working surfaces.
- ✓ Routinely check the drip pan beneath the work surface for cleanliness, and if a spill has occurred, clean and disinfect it.
- ✓ Take care to prevent towelettes from being sucked into exhaust plenums.
- ✓ If the BSC is known to have Ready Safe mode, sash can be closed without turning off the BSC blower. Otherwise, turn off the BSC blower before closing the sash.
- ✓ The drip pan below the work surface should be periodically decontaminated and cleaned even in the absence of a spill.

### **Activities That Can Compromise Protection**

The purpose of using a BSC is to protect users and the environment from hazardous biologicals used, and to protect samples from contamination. Protection can be compromised by a number of ways:

- **Improper sash height** alters the inward air velocity. Sashes lowered too far can cause the inward air from the room to reach the clean work surface whereas when sashes are raised too far, contaminated air can escape the BSC. Always use the sash at the height as indicated by the manufacturer.
- **Items on the front grille** diverts the inward air to the clean work surface. Never place items including bottles and paper on the grille nor tape autoclave bags to the grille. Arms should also not rest on the grille while working.
- **Foot traffic** has the potential of disrupting the protective air curtain, causing dirty air to enter the BSC and contaminated air to exit the BSC. BSCs should be located in the room such that nearby foot traffic is minimized. Whenever walking past a BSC, always walk slowly and with the greatest distance between you and the BSC.
- **Doors** near the BSC can disrupt the protective air curtain when they are opened and closed. This includes room doors but also doors to refrigerators, freezers, and incubators that are frequently placed next to the BSC. If a piece of equipment is next to the BSC, place it such that any doors open away from the BSC and there is a minimum of 6 inches between the equipment and the BSC.
- **More than one person** working in a BSC leads to cross contamination in all BSCs regardless of size and sash height. All protection including personnel protection can be lost when more than one person is working in a BSC with a 12 inch sash height.
- **Equipment** used inside a BSC can disrupt the air flow by diverting the downward laminar air flow, increasing the risk of cross-contamination and loss of containment. A smoke test needs to be performed with the equipment in the BSC to confirm that containment is not compromised. The BSC Certification Program can perform the smoke test upon request.
- **Animal cages** in a BSC have a similar affect as equipment by diverting the downward laminar air flow. The number of cages and the height of the stack should be minimized.
- **Heat-generating devices** disrupts air flow patterns and causes air turbulence. Please see the below section 'Use of Open Flames or Heat Sources in BSCs' for more information.
- **Sweeping arm movements** are very disruptive to the protective air curtain, causing dirty room air to reach the clean work surface or contaminated air to escape from the BSC. Always move hands and arms straight into and out of the work area.
- **BSCs with unbalanced airflows** might not provide personnel and product protection. The downward airflow from the supply HEPA filter needs to be balanced with inward air flow from the room to generate the protective air curtain.

If the downward airflow is greater than the inward air flow, contaminated air can escape the BSC. Whereas if the inward air flow is greater than the downward air flow, dirty room air can reach the clean work surface. BSC certification ensures that these airflows are balanced.

### Potential Sources of Contamination

Contamination inside a BSC can derail experiments and ruin samples. There are numerous sources of contamination including:

- **Dirty lab coats** are a common source of contamination. Using lab coats dedicated to the BSC, frequent laundering, or using disposable lab coats is helpful.
- **Sloppy lab practices** can cause contamination. This includes placing items on the grille, sweeping arms inside and out of BSCs, failing to decontaminate items prior to placing inside the BSC, placing dirty items next to clean items, and using a workflow that creates a constant disruption of the air curtain. It is always best to work with smaller waste containers inside the BSC to reduce the frequency of crossing the air curtain and to place these containers away from clean items.
- **Items from water baths** have been found to be a source of contamination. Always decontaminate surfaces of items prior to placing them inside the BSC.
- **More than one person** working in a BSC leads to cross contamination inside the BSC and may also compromise product protection. Regardless of the BSC size, allow only one person to work in the BSC at a time unless it is necessary for the experiment.
- **Clutter** disrupts air flow patterns and diverts the downward laminar air flow, causing cross-contamination. Limit items inside the BSC to just those required for that procedure.
- **Equipment** also disrupts air flow patterns and diverts the downward laminar air flow. A smoke test should be performed to confirm that containment has not been compromised. However, the risk of cross-contamination can not be eliminated when equipment is used inside a BSC.
- **Heat-generating devices** can cause turbulent air flows, leading to problems with cross-contamination. Use alternatives that do not generate heat such as disposable inoculating loops.
- **Improper starting and shutting down** of BSCs does not establish or maintain a clean environment inside the BSC. Always wait at least 10 minutes after turning on the motor/blower to provide sufficient time for the BSC to purge contaminants from the BSC. When shutting down the BSC after use, the best practice depends on the BSC model. If the BSC has Ready Safe mode, the best practice is to leave the motor/blower on with the sash closed. This will maintain a clean environment. If the BSC does not have Ready Safe mode, the best practice is to either leave the motor/blower on with the sash open or turning the motor/blower off and closing the sash. The BSC can not maintain a clean environment if the motor/blower is turned

off, thus it is important to use a disinfectant inside the BSC when preparing to use it.

- **BSCs with unbalanced airflows** may not prevent dirty room air from entering the BSC. Moving BSCs can imbalance airflows. Always have the BSC certified annually and after moving so that air flows can be re-balanced for maximum protection.

### **Addressing Contamination Problems**

If a BSC is experiencing a contamination problem, use the list above as a starting point for identifying the potential source of the contamination. Gas decontamination of a BSC is expensive and most labs who have chosen to go this route, it did not eliminate their contamination problem as the source of the contamination was not the BSC itself. Dirty lab coats, sloppy lab practices, and dirty items from the water bath are the most common source of contamination. If you want the BSC to be gas decontaminated, please contact the BSC Certification Program (608-262-1809; [bscservices@fpm.wisc.edu](mailto:bscservices@fpm.wisc.edu)).

### **Procedures in the Event of an Alarm**

The purpose of alarms is to alert users when there are conditions that could compromise protection. These conditions can be improper sash height, power failure, or reduction of building exhaust.

The sash height is important to establish and maintain protective airflow patterns within the BSC. For each BSC, the manufacturer will indicate the correct sash height. At incorrect sash height, the BSC will alarm to indicate increased risk to personnel and samples. In the event of a sash alarm, do not silence it but rather adjust the sash to the correct height. Once the alarm ceases, work can resume.

An alarm will sound in the event of power failure because the motor/blower cannot operate to maintain the protective airflow patterns. All levels of protection are lost during a power failure. If this occurs, stop all work and close all containers. Return materials to their storage location if power does not resume after a short period of time. Work can resume once power is restored. If loss of power is due to a BSC failure, contact the BSC Certification Program (608-262-1809; [bscservices@fpm.wisc.edu](mailto:bscservices@fpm.wisc.edu)) to schedule repairs. Do not use the BSC until it is repaired.

BSCs may be connected to the building exhaust system to exhaust volatile chemicals or gases used inside the BSC. An alarm will sound when there is a loss or reduction in the building exhaust. In such situations, the motor/blower can pressurize the inside of the BSC causing a loss of containment and an exposure to the users as air is blown towards users. All Class II Type B BSCs are directly connected to the building exhaust system and newer models have an interlock mechanism in which the motor/blower is simultaneously turned off upon alarming to prevent pressurization of the BSC. Older models do not have this safety feature. For Class II Type A2 BSCs which have a FlexAir canopy connection to the building exhaust system, the canopy prevents pressurization by exhausting HEPA filtered exhaust air into the room since it can not exhaust through the

building exhaust system. This air is HEPA filtered so the risk of an exposure to biologicals used in the BSC is low. However, if volatile chemicals or gases are in use, people in the room can be exposed. For both BSC types, if the BSC alarms due to the building exhaust system, stop all work, close all containers, and return materials to their storage location. Contact your building manager so that they can determine if the building exhaust system is the cause of the alarm. A work order will likely need to be placed so that Physical Plant can adjust the building exhaust intake. Work must not resume until the BSC Certification team deems it is safe to do so.

### **Use of Disinfectants in a BSC**

Most disinfectants are corrosive to the metal surfaces of a BSC. Some are less corrosive such as Cavicide. The use of ethanol is popular since it is not corrosive. However, if your lab has an approved biosafety protocol, its use as a sole disinfectant needs approval before ethanol can be used. In general, ethanol evaporates quickly, not allowing for sufficient contact time. Therefore, the use of ethanol by itself for disinfection is not recommended. Instead, it is recommended to use ethanol to remove residual disinfectant from the metal surfaces to help prevent corrosion. Regardless, ensure the use of a disinfectant that is effective against the agents (e.g. microbes, cells) you are working with and that the disinfectant used is listed in your approved biosafety protocol if your lab has one.

### **Decontamination and Spills**

Spills within the cabinet should be cleaned up according to the spill protocol found inside the lab's approved biosafety protocol. If the lab's research does not require a biosafety protocol, please contact OBS for assistance in developing a spill protocol. Of note, spills large enough to result in liquids flowing through the front or rear grilles require more extensive decontamination. All items within the cabinet should be surface decontaminated and removed. Beneath the BSC work surface is a drip pan to collect large spills. After ensuring that the drain valve is closed, decontaminating solution can be poured onto the work surface, grilles, and the drain pan. Twenty to thirty minutes is generally considered an appropriate contact time for decontamination, but this varies with the disinfectant and the microbiological agent. The drain pan should be emptied into a collection vessel containing disinfectant. If the drain pan is accessible, wipe it down to remove remaining debris. Should the spilled liquid contain radioactive material, Radiation Safety personnel should be contacted for specific instructions on conducting a similar procedure.

### **Use of ultraviolet (UV) lights in BSCs**

UV lights in BSCs have been used for their germicidal property. However, their use in BSCs is neither recommended nor required by the CDC and the NIH. The Office of Biological Safety does not approve the use of UV lights in BSCs as the primary means of decontamination.

The germicidal benefit of UV lights is limited by several factors. UV light does not penetrate and therefore, surfaces that are covered by items or in shadow are not decontaminated. Dust and fingerprints on UV bulbs can also block UV lights.

Temperature, relative humidity, and air movement all affect the efficacy of UV lights. Lastly, the intensity of the UV light decreases with age and use. UV bulbs require more frequent cleaning, maintenance, monitoring, and replacement than is typically provided by users.

The use of UV lights poses a risk to humans. Exposures to skin can cause erythema, a reddening of the skin similar to sunburns. Exposures to eyes can cause photokeratitis and/or conjunctivitis. The effects from UV radiation can vary depending on wavelength, the level of exposure, and the duration of exposure. UV light does not penetrate the BSC sash and thus, always keep the sash fully closed when the UV light is used. In fact, most BSCs have a safety feature that prevents UV lights from being used when the sash is not fully closed.

New BSCs no longer come standard with UV lights installed. Even though the use of UV lights is not recommended, UV lights may be installed in a BSC by either the BSC manufacturer or the UW-Madison BSC Certification Program. Any UV light fixtures installed by a different party including the lab will not be allowed. In such instances, there can be an increased risk of exposure to UV radiation. In addition, the fixture can compromise containment and protection by affecting the BSC air flow patterns.

\*Please remember that in newer BSC models, operating in Ready Safe mode when the BSC is not in use will provide better protection than the use of UV lights since it can maintain personnel, product, and environment protection while in idle.

### **Use of Open Flames or Heat Sources in BSCs**

Heat from Bunsen Burners and other heat-generating devices (e.g. Bacti-Cinerator, bead sterilizer) can affect the air flow in BSCs and as a result can compromise user safety and increase contamination. Held, *et al.* (Held, K. F., Thibeault, R., & Boudreau, J. (2019). "Heat Sources in a Biosafety Cabinet Compromise Experimental and User Protection". *Applied Biosafety*, 24(2), 90–95. <https://doi.org/10.1177/1535676019831173>) studied the effects of heat on BSC air flow patterns. Smoke studies revealed that the heat disrupts air flow patterns and causes air turbulence. When low heat sources such as alcohol lamps and Bacti-Cinerator were used, the BSC could still prevent dirty room air from entering the BSC work surface but found that personnel protection may be lost, and cross-contamination occurred. When Bunsen Burners were used, BSCs could not prevent dirty room air from entering the BSC work surface, personnel and product protection were lost, and cross-contamination occurred. It was also found that 4-foot wide BSCs had greater difficulties than 6-foot wide BSCs in overcoming the heat disturbance. The placement of the heat source within the BSC was also studied and found that the most common location of the heat source (the center of the work surface) was prone to the most failures. For these reasons, the use of heat-generating devices in a BSC may not in fact be providing you with the level of protection you need.

Besides disrupting air flow patterns, excessive heat build-up can also damage the HEPA filters by damaging the media and the adhesive used for the HEPA filters (figure 8). Replacing HEPA filters can cost several thousand dollars.

**Figure 8. Damaged HEPA filter caused by a Bunsen burner.** Photograph courtesy of Con-Test.



The use of flammable gas inside a BSC is an explosive hazard (figure 9). Fires and explosions can occur by ignition of built-up gas. The build-up of gas inside a BSC can occur if a shut-off valve is inadvertently left on, when the flame is blown out by the turbulent environment, or from faulty tubing, connections, or shut-off valve. Even though flammable

gas can be plumbed to the BSC, BSC manufacturers do not approve this practice. They approve the use of service valves in BSCs with non-flammable gasses and liquids (e.g. CO<sub>2</sub>, house air, vacuum) only.



**Figure 9. BSC destroyed when flammable gas used inside the BSC ignited.**

Due to the risk of using Bunsen Burners and heat-generating devices inside a BSC, safer alternatives should be used when possible. The guidance document *Bunsen Burner Alternatives* found on the UW-Madison EH&S website can provide more information regarding alternatives. As of October 2019, when procedures require a flame or heat-generating device in a BSC, their use must be approved through the Office of Biological Safety. The approval process will include justification on the use of the device, a risk assessment, parameters set by OBS on the use of the device, and additional training. Please contact the Office of Biological Safety if you would like to use a heat-generating device in a BSC or if you are currently using a heat-generating device in a BSC without OBS approval.

### **BSC training**

There are many factors and activities that can compromise the protection provided by a BSC. Thus, users should be trained on how to properly use a BSC by experienced users. In addition, on-line BSC training is available through Canvas. A list of all required and optional safety trainings offered by Environment, Health, and Safety including the on-line BSC training can be found at <https://ehs.wisc.edu/ehs-training-overview/>. In-person BSC training with the BSC liaison in the Office of Biological Safety can be requested by emailing [biosafety@fpm.wisc.edu](mailto:biosafety@fpm.wisc.edu).

### **BSCs, ATSSs, and CADs POLICIES AND REQUIREMENTS**

The purpose of Institutional Biosafety Committee (IBC) BSC policy ([IBC-POL-18](#)) is to provide standards for the UW-Madison research community on the certification, use, and maintenance of biological safety cabinets, animal transfer stations, and clean air devices.

This policy covers the purchasing or acquisition, certification and use, maintenance and service, relocation within campus and off campus, disposal, and the use of heat-generating devices in these types of equipment. All BSC users should read and be familiar with this policy.

In regard to BSCs, ATSSs, and CADs, the following elements are found within the IBC BSC Policy:

**Purchasing**

**Acquisition of Used Equipment**

**Certification and Use Requirements**

**Waivers from Certification**

**Equipment Lock-outs**

**Relocation Within the UW-Madison Campus**

**Lab Transfer from One Institution to Another**

**Disposal**

**Decontamination Requirements**

**Approval for Open Flame Use in BSCs**

**Room Placement**

For proper function, containment equipment should be placed in a location with minimum disruption of its airflow patterns. The ideal placement is away from doors, room supply air registers, fans, other containment equipment (e.g. fume hood), and foot traffic. Doors to rooms, incubators, refrigerators, and freezers should open away from BSCs. There should be a minimum of 6 inch clearance to the sides of the BSCs and 6 inch clearance to the top of the BSC.

**Utility Requirements**

BSCs, ATSSs, and CADs require a dedicated 20 A, 115 V, one phase, 60 Hz circuit and no other equipment shall share this circuit. The wall receptacle must be rated for 20 A, 115V and fit a NEMA5 plug. In addition, the wall receptacle must be accessible for visual inspection and located such that the plug can be removed without moving the BSC or any other lab equipment.

**BSC Modifications**

Modifications to BSCs can impair their functionality and the protection they provide. Holes shall not be drilled into BSCs. Light fixtures that are not installed by the manufacturer or the BSC Certification Program are not permitted. Any unauthorized modifications will require the purchase of parts to return the BSC to original build at the expense of the party performing the unauthorized modification.

**SERVICES PROVIDED BY THE UW-MADISON BSC CERTIFICATION PROGRAM**

## **Certification Process**

To ensure adequate function of BSCs, ATSS, and CADs, airflows are balanced, HEPA filters are tested, and other tests (e.g. alarms, sensors, lights) are performed during certification in accordance to NSF Standard 49. Certification requirements and frequency is defined in the IBC BSC Policy ([IBC-POL-18](#)). The owner of the equipment is responsible for all expenses associated with the it (e.g. certifications, repairs, decontamination, disposal). The BSC Certification Program will contact the owner or contact person to schedule certification. If the BSC is moved, it must be certified before use and it is the responsibility of the owner to schedule this certification. It is prudent to contact the BSC Certification Program greater than a month prior to moving so that the unit can be disinfected prior to the move and certified shortly after the move.

During certification, if balanced airflows cannot be achieved, the HEPA filter does not pass an integrity test, or the unit is found unsafe to operate, the certification will be failed. If this occurs, the BSC Certification Program will email the owner or contact person to explain the reason for failure and what the next steps are. A BSC or ATS cannot be used after a failed certification until it has been repaired and certification. Only the Office of Biological Safety or the Institutional Biosafety Committee can determine whether a failed unit can be used until repaired.

For those BSCs which are connected to the building exhaust, adjustments to the building exhaust may be needed in order to achieve balanced air flows. If these adjustments are needed, the building manager will need to generate a work order with FP&M so that FP&M personnel are present to make adjustments during the certification.

## **Maintenance and Repairs**

Common repairs needed include replacing bulbs, ballasts, HEPA filters, motor, and speed controller. If your unit needs repairing, contact the BSC Certification Program (608-262-1809; [bscservices@fpm.wisc.edu](mailto:bscservices@fpm.wisc.edu)). To speed up the process, please submit the online request at <https://ehs.wisc.edu/bsc-certification-maintenance-and-service-requests/> and be sure to complete all sections. If the BSC Certification Program must access the contaminated plenum of a BSC, the unit must be gas decontaminated first. The owner is responsible for all costs associated with the repairs including gas decontamination if one is needed.

## **Decontamination**

The BSC Certification Program may perform surface decontaminations when BSCs are moved. The units are also wrapped to protect the BSC and the movers. The BSC Certification Program also performs gas decontaminations when BSCs are moved, repaired, or dismantled/disposed. During gas decontaminations, only employees of the BSC Certification Program are permitted to occupy or enter the room in which the BSC is located.

### **How to Set Up an EH&S Cost Center**

Instructions on how to set up an EH&S Cost Center can be found on the EH&S website at <https://ehs.wisc.edu/biological-safety-cabinets/>.