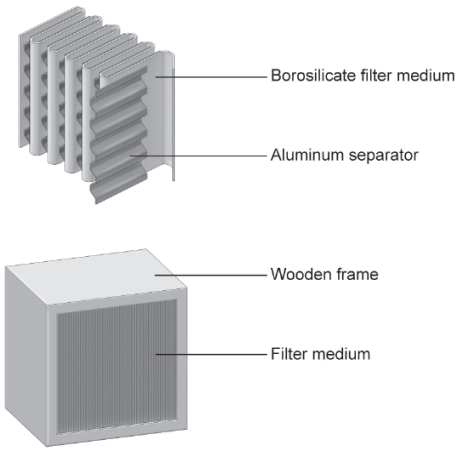
Appendix 3.2.a: CONTAINMENT DEVICES (ENGINEERING CONTROLS)

Engineering controls are physical devices designed to isolate hazards and minimize the risk of exposure or release within the laboratory. Biosafety cabinets (BSCs), centrifuge safety cups, and safe needle devices are among the more common engineering controls used for work with infectious agents.

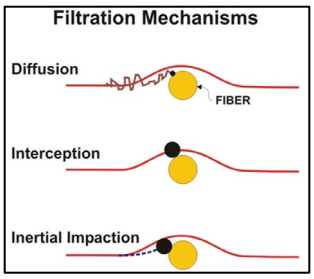
A) Biosafety Cabinets



BSCs are primary containment devices that utilize mechanical air filtration and directional airflow to protect personnel and the environment from exposure/release of the materials being worked with (product) in the cabinet. Most BSCs also protect the product itself, by reducing the risk of cross-contamination of samples or contamination from outside air. At UAB, BSCs are required for work with any RG2 agent, if the work is reasonably anticipated to generate aerosols or droplets. To aid in the understanding and appropriate use of BSCs, an extensive overview of available models, mechanisms, and applications are included below. For more detailed information, refer to Appendix A of the BMBL 6, or call EH&S.

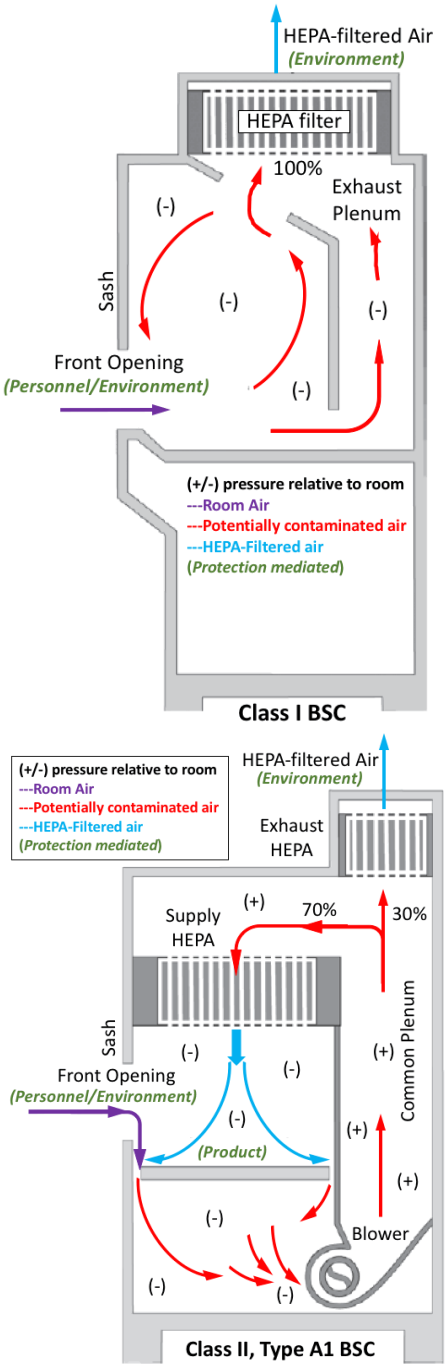
Components of a Biosafety Cabinet:

- **High Efficiency Particulate Air (HEPA) filters:** BSCs rely on specialized HEPA air filters to mediate their protective functions. HEPA filters are defined by their ability to remove 0.3 μm particles at 99.97% efficiency. Other particle sizes are also efficiently removed by HEPA filtration, but the 0.3 μm sized particles are the most difficult to exclude and thereby serve as the metric for defining a "HEPA filter." HEPA filters are typically constructed of pleated mats of randomly arranged borosilicate fibers that are coated with a water-repellant binder. The pore size between these fibers is typically much greater than 0.3 μm, so filtration is achieved through interception, impaction, and diffusion. The filter fiber diameter, thickness of the filter, and the face velocity all directly affect the efficiency of these mechanical filtration mechanisms.
    - **Interception:** is the process by which particles passing within a certain distance of a fiber are sequestered from the air through adherence
    - **Impaction:** larger particles are unable to avoid contact with the filter media and are sequestered by impaction with a fiber.
    - **Diffusion:** describes the movement of much smaller particles, which are affected by gas molecules, resulting in a disordered path through the medium and an increased likelihood of interception or impaction.



- **Directional airflow:** mechanical blowers and pressure differentials drive the directional movement of air currents in a BSC. The front grill of the BSC draws in a mixture of room air and potentially contaminated air from the work surface. This establishes an air "curtain" that prevents aerosols generated in the cabinet from exiting through the front opening. In some BSCs, supply

air is drawn from both the room and from recirculation from the front and rear grills. This supply air passes through a HEPA filter above the cabinet work surface and moves downward before splitting horizontally to the front and rear grills. This laminar airflow serves to protect the product from contamination. Any air leaving a BSC must first pass through a HEPA filter before being exhausted, which serves to protect the environment, if exhausted outdoors, or both personnel and the environment, if exhaust air is recirculated into the laboratory. Different BSCs offer varying degrees of personnel, environmental, and product protection, and are categorized based on the position of the HEPA filter(s), airflow patterns and velocities, and exhaust/recirculation routes.

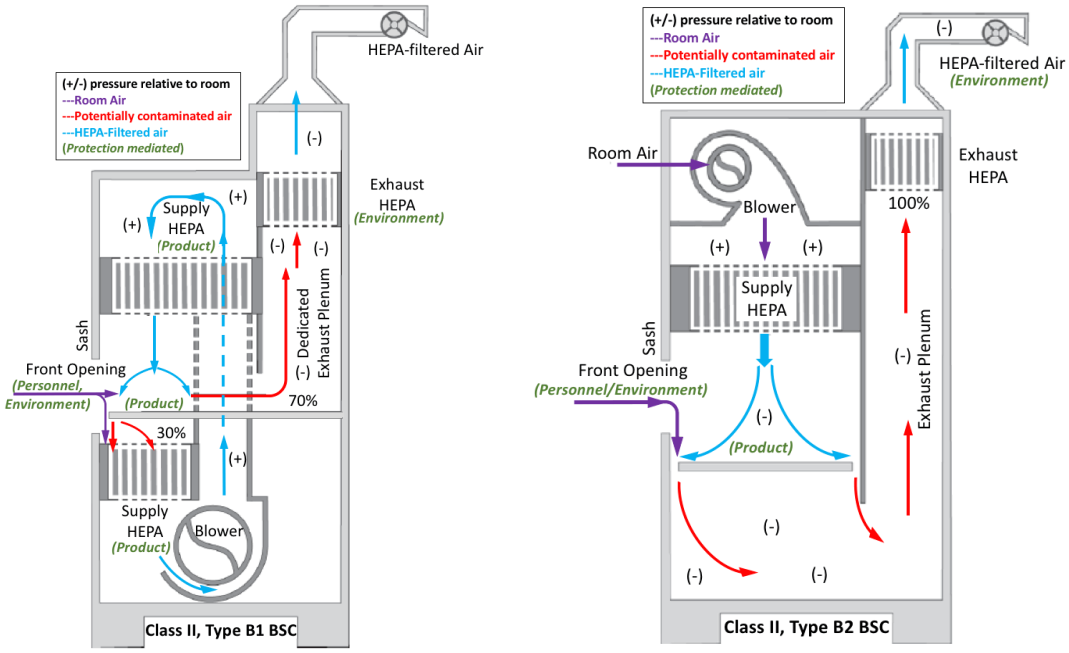


Class/Types of BSCs

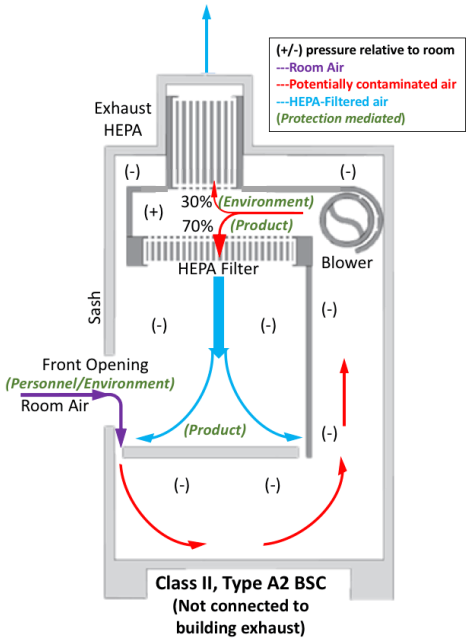
Class I BSCs: Class I BSCs protect the environment and personnel but offer **no product protection**. Environmental protection is achieved through HEPA filtration of exhaust air and personnel protection is achieved by the inward directional movement of unfiltered air through the work opening. These devices are typically used to enclose equipment that has the potential to generate aerosols.

Class II (Types A1, A2, B1, and B2) BSCs: Class II BSCs offer personnel, environmental, and product protection. Personnel protection is achieved by the inward flow of unfiltered air into the front grill. Product protection is achieved by the downward and split-directional laminar flow of HEPA-filtered air into front and rear grills, and environmental protection is achieved via HEPA filtration of air recirculated into the Laboratory (Types A1 and A2 BSCs), discharged via a canopy or thimble connection to the building exhaust (Types A1 and A2 BSCs), or hard ducted exhaust (Types B1 and B2 BSCs).

- **Class II, Type A1:** These BSCs have two HEPA filters, with 70% of air recirculated through the supply HEPA filter back into the BSC work zone, and the remaining 30% of air passed through an exhaust HEPA filter, to be recirculated into the laboratory. Due to potential buildup of toxic vapors from air recirculated into the lab and cabinet, volatile toxic chemicals are not to be used in these BSCs.
- **Class II, Type B1:** The design of these cabinets allows for small quantities of hazardous chemicals to be used. This is due to the fact that 70% of the air flowing downward to the work surface exits through the rear grill and is discharged from the building. The remaining 30%, as well as external room air, is drawn through the front grill and passes through a supply HEPA filter before it is returns across the work surface. Since the air entering the back grill is exhausted, hazardous chemical work should be conducted toward the rear of the cabinet.



- **Class II, Type B2:** These cabinets do not recirculate; all air is exhausted after passing through a HEPA filter. Supply air is drawn through the front grill, HEPA filtered, and passed across the work surface. Volatile chemicals can only be used if the vapors are compatible with the filter medium. Since the exhaust is hard ducted to the building exhaust, the cabinet will become positively pressured if the building exhaust fails. Interlocks are used to shutoff the supply blower when building exhaust rates are insufficient, and flow meters are used to warn users of a failure.
- **Class II, Type A2 (formerly A/B3):** Compared to Class II/A1 BSCs, which may have positively-pressured, biologically contaminated plenums, the plenums of these BSCs are under negative pressure to room or surrounding cabinet envelop, adding additional personnel protection in the event of a leak.



Types of Class II Biosafety Cabinets			Type A1	Type A2	Type B1	Type B2
Containment & Protection	Particulate Protection	Personnel	✓	✓	✓	✓
		Product	✓	✓	✓	✓
		Environment	✓	✓	✓	✓
	gas/vapor Protection	Personnel	X	If exhausted through facility	✓	✓
		Product	X	X	Reduces exposure	✓
		Environment	X	If exhausted through facility	Reduces exposure	If exhausted through facility
Airflow	Cabinet Face Velocity		≥75 FPM	≥100 FPM	≥100 FPM	≥100 FPM
	Nominal %	%Recirculated	70	70	30	0
		%Exhausted	30	30	70	100
Plenum	Biologically Contaminated plenum pressure		Pos. to room	Neg. to room or surrounded by neg. pressure	Neg. to room or surrounded by neg. pressure	Neg. to room or surrounded by neg. pressure
Exhaust Properties	Cabinet Exhaust Source		Common plenum	Common plenum	Exhaust plenum	Exhaust plenum
	Exhaust Destination	To room	✓	✓	X	X
		Vented outside	Optional	Optional	✓	✓
		Connection type	canopy	canopy	Hard ducted	Hard ducted

Proper Use of a BSC:

Pre-work checklist:

- Don (or put on) the appropriate PPE. The PPE should be determined by your protocol.
- If turned off after each use, decontaminate work surfaces exposed to room air
- Airflow and possible contamination will be lower if you do not have to move in and out. Therefore, load the supplies first.
- Turn the biosafety cabinet on and allow it to run for 5 minutes before use.
- Check the inward airflow by securely attaching a piece of tissue to the face hood. The tissue should be pulled in toward the cabinet.
- Make sure the sash is at the certification levels posted on the BSC.
- Adjust seat height so that the bottom edge of the sash is level with your underarms.

Working in the BSC:

- Always designate a clean side and a dirty side. Work from clean to dirty, and work on centerline of work surface. Note the location of discard trays and how other items are positioned to avoid compromising the airflow.
- Work on the approximate centerline. This is the recommended best location to maintain the integrity of proper airflow.
- Move slowly and deliberately into and out of the biosafety cabinet. Slow and deliberate movement has very little effect on the airflow, but rapid and sudden movements can disrupt the airflow dramatically causing issues with contamination.
- Avoid blocking the front grill. When front grill is blocked, airflow can be disrupted. Blocking the front grill also allows the room air to enter the biosafety cabinet.
- Place lab supplies and materials inside the biosafety cabinet. Place them in a location where the airflow is not disrupted.

Post-work Checklist:

- Disinfect all of the items to be removed from the cabinet
- Remove all waste products and place in appropriate receptacles
- Wipe down the interior of biosafety cabinet with an appropriate disinfectant
- Allow cabinet to run for 10 - 15 minutes before shutting off
- If you are using a UV light, make sure you still follow proper procedures. A UV light will not destroy all microbes, so an appropriate disinfectant must be used. UV lights should be wiped down at least once per week when the light is off.

BSC Care and Maintenance:

- **Decontamination:** Surface decontamination of a BSC is conducted using a disinfectant that is active against the agents being used. Chemical compatability with the stainless steel surfaces is also a concern. If corrosive disinfectants are used, the surfaces are typically rinsed with water following the appropriate contact time with the disinfectant. Full decontamination of a biosafety cabinet is not performed on a regular basis, as it typically requires paraformaldehyde gas or vaporized hydrogen peroxide (VHP) to ensure the HEPA filter is thoroughly disinfected and these chemicals pose their own exposure risks. BSCs that have only been used for product protection (i.e., no biohazardous work) do not need to be disinfected. A full decontamination is required for the following situations:

  • The BSC was used for work with biohazardous materials and it is being decomissioned or moved to the surplus warehouse
  • The BSC was used for work with biohazardous materials and repair work is needed that may expose service technicians

  BSCs are rarely a source of sample contamination unless the filter is not working properly (fails certification). Requests for in-house BSC decontamination services to address sample contamination issues will receive low priority until a consult with Biosafety has been conducted. Biosafety representatives will work with laboratories to ensure all other potential sources of contamination are addressed before recommending decontamination of a BSC (e.g., contaminated refrigerators, incubators, and waterbaths, or poor sterile technique).

  Certification: Class II biosafety cabinets are regulated by the National Sanitation Foundation (or NSF). The certification procedures listed here have been mandated by NSF and the NIH. Certification procedures assure the user that the protection factors of personnel, product, and environment are maintained by verifying that the down flow velocities, in-flow velocities, and HEPA filters are within specification. EH&S representatives are responsible for BSC certification across campus, upon request. Contact EH&S at biosafety@uab.edu to schedule an appointment or make inquiries.

  BSCs must be recertified:
  - After the unit has undergone repairs that necessitate re-certification
  - After a unit has been installed or relocated
  - At least annually thereafter

  The IBC may require work with specific agents (RG2 or higher) to only be conducted in a BSC. In this case, the BSC certification must be valid for active work to continue. If EH&S representatives find a BSC with an expired certification, the PI will be notified, and a sticker will be placed on the BSC indicating it is not to be used for personnel protection. Continued

use of an uncertified BSC for personnel and/or environmental protection is reportable to the IBC.

Animal cage change station:

Animal cage change stations are used to reduce animal allergen exposures to users, ARP staff during cage changes or normal animal husbandry practices. Animal cage change stations should not be used for cage changes and animal husbandry practices at ABSL-2 and higher (work with infectious agent or biological hazard or chemicals) due to an increased risk of exposure to users, ARP staff and maintenance personnel. Cage changing of infectious agent administered animals must be performed inside Biosafety cabinet. Follow Animal Use Safey Information (AUSI) guidelines recommended by Institutional Biosafety Committee. Animal cage change stations must be certified annually.

B) Centrifuge Safety

Many researchers are conscientious of the physical hazards associated with centrifugation, but fail to realize the potential risk of creating infectious aerosols. Engineering controls have been devised to accommodate centrifugation of infectious samples, but there are important safety processes to consider before, during, and after centrifugation, outlined below.

Before centrifugation

- Train all potential users on proper operating procedures, review the user manual.
- Use only rotors compatible with the centrifuge. Check the expiration date for ultracentrifuge rotors.
- Check rotors, bottles, and tubes for cracks/deformities before use.
- Clean and dry the rotor, tubes, and spindle after use.
- Examine O-rings and replace if worn, cracked, or missing.
- Never overfill centrifuge tubes (don't exceed 75% capacity).
- Always cap tubes before centrifugation.
- Always balance buckets, tubes, and rotors properly.
- Check that the rotor is seated on the drive correctly, close the lid on the centrifuge, and secure it.
- When using swinging bucket rotors, make sure that all of the bucket positions are filled and correctly hooked to move freely.

During centrifugation

- Keep the lid closed at all times during operation. Never open a centrifuge until the rotor has stopped.
- Do not exceed safe rotor speed.
- The operator should not leave the centrifuge until full operating speed is attained and the machine appears to be running safely without vibration.
- Stop the centrifuge immediately if an unusual condition (noise or vibration) begins and have user check load balances.

After centrifugation

- Allow the centrifuge to come to a complete stop before opening.
- Wear gloves to remove rotor and samples, see Glove Selection and Use.
- Check inside of centrifuge for possible spills and leaks, clean centrifuge and rotor thoroughly if necessary.
- Wash hands after removing gloves.

Centrifuging infectious materials or potentially-infectious samples

Safety procedures above, plus:

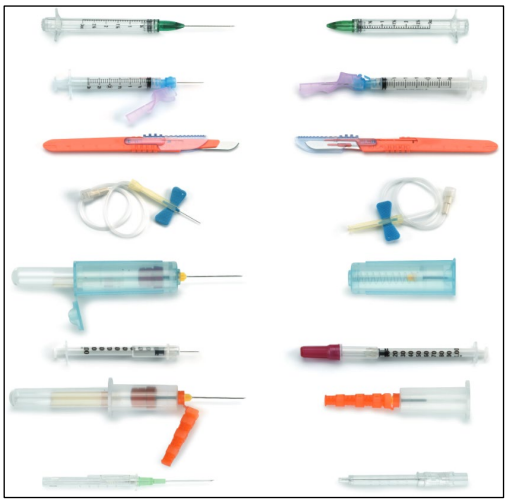
- Place a biohazard label on the centrifuge.
- Include centrifugation procedures and decontamination plans in lab SOPs.
- Always wear gloves when handling tubes or rotors.
- Avoid the use of celluloid tubes with biohazards. If celluloid tubes must be used, an appropriate chemical disinfectant must be used to decontaminate them.
- Always use sealed safety cups, safety buckets, or sealed rotors with O-ring as secondary containment, if available, or required by IBC.
- Fill centrifuge tubes, load into rotors, remove from rotors, and open tubes within a biological safety cabinet. Wipe exterior of tubes or bottles with appropriate chemical disinfectant prior to loading into rotor or bucket. Seal rotor or bucket, remove outer gloves, and transport to the centrifuge.
- If sealed safety cups are not used, wait at least 10 minutes after the run to allow aerosols to settle before opening the centrifuge. Check for possible spills or leaks. For spills of infectious materials, see Lab-specific Safety Plan for response procedures.
- Decontaminate centrifuge interior, safety cups or buckets, and rotors if tube breakage occurs. See Lab-specific Safety Plan for response procedures.

Centrifuge Maintenance

Moisture, chemicals, strong cleaning agents, and other substances can promote corrosion of centrifuge parts and cause centrifuge failure. The following are general maintenance recommendations:

- Follow manufacturer instructions for maintenance and cleaning.
- Keep the centrifuge clean and dry.
- Cleanup all non-infectious spills immediately. Lab-specific Safety Plan for response procedures.
- Decontaminate the rotor after use with biological materials.
- Never clean rotors and associated parts with abrasive wire brushes.
- Store the rotor upside down in a dry place, with lids or plugs removed, to prevent condensation.
- Remove adapters after use and inspect for corrosion.
- Inspect rotor regularly. Remove rotors from use that show any sign of defect, and report it to a manufacturer's representative for inspection.
- For high-speed rotors, maintain a log book to track the speed and spin time for each use, and discard rotors according to the manufacturer’s recommendations.

C) Engineering Controls for Sharps



“Sharps” is a broad term to describe any object capable of causing percutaneous injury, including but not limited to, needles, scalpels, microscope slides, capillary tubes, Pasteur pipettes, scissors, and broken glass or plasticware with sharp edges. Biomedically-contaminated sharps have the added infection and health risk to both the user and others who may come in contact with them before final disposal. Whenever possible, administrative policies should seek to eliminate or reduce the use of sharps (e.g., substitution of glassware with platicware). When sharps are required for infectious disease research, specialized devices that reduce the risk of percutaneous injury/exposure may be available. These engineering controls include needleless systems, retractable/self-sheathing needles or scalpel blades, and disposable scalpels.

The containers used for disposing of contaminated sharps are also a form of engineering control. Only approved sharps containers can be used for disposing of biomedical sharps waste. These containers must be:

- leak-proof
- non-breakable
- rigid
- puncture-resistant
- autoclavable
- chemically resistant, as required
- adequate to contain the sharp items
- labelled with the appropriate warning logo
- secured with a non-removable lid that does not allow access to the disposed material is preferred.
- disposed as biohazardous waste through UAB waste vendor.

Consult EH&S at biosafety@uab.edu if you have questions about sharps safety controls or disposal.

D) Aerosol Management Systems for Fluorescence Activated Cell Sorting (FACS)

Flow cytometers are the instruments that provide measures of the quantitative properties of single cells, one cell at a time. Cytometers can evaluate cell size and the fluorescence properties of cells by creating droplets. Based on the scatter and florescence properties, the populations of cells of interest, can be sorted into a sample receptacle by applying a charge to the droplets. Specimens analyzed through cell sorting core facilities can be broadly sourced and may contain known or unknown pathogens from human or animal sources. During the sorting process, pressurized liquid samples create droplets of cell suspension. These droplets are generated in large concentrations. Data suggests droplets are within the respirable size range and may be associated with infective pathogens. Based on these factors, safety procedures should be adopted in order to mitigate the risks of exposure to cell sorter operators and other laboratory workers.

Safety recommendations outlined in SECTION 3 PRINCIPLES OF BIOSAFETY: BSL2 (including all PPE, primary barriers, facility enhancements) should be followed for laboratories performing cell sorting. Since it is not possible to test cell lines for every possible human pathogen or to assert that they are pathogen-free, and due to the potential for aerosol exposure during cell sorting, human cell lines should be sorted using biosafety precautions appropriate for human blood and body fluids, i.e. BSL2. Ideally, flow cytometry and cell sorting equipment should be housed in an annually certified BSC. However, due to cost and BSC size limitations, alternative means of containment are often implemented for use with cell sorting devices.

Many devices utilize aerosol management systems, to aid in the containment of aerosols, by evacuating the sort collection chamber. These systems use vacuum attachments to rapidly evacuate aerosols through am ultra-low penetrating air filter (99.99% efficiency down to particles 0.1 microns in size) during routine sorting or analysis. Care should be taken to regularly check

vacuum lines, pumps, and filters. Loss or degradation of any component may result in a positively pressured sorting chamber and lead to a potential environmental (laboratory) release of aerosols.

Laboratory managers or PI's (including core facilities) should maintain containment devices according to manufacturer suggestions. Special attention should be paid to any indicated filter malfunctions or expirations. Annual safety audits may be conducted by EH&S at any core facility. Please contact EH&S Biosafety at biosafety@uab.edu for questions or assistance.