Biosafety Basics

Module 3: Containment

Material Covered

- Definition of Containment
- Aerosol and Droplet Generating Laboratory Activities
- Biological Safety Cabinets (BSCs)
  - Operation
  - Best Practices
  - Certification
- HEPA Filters
- Safety Centrifuges
- Flow Cytometry

Containment

- Primary containment is the protection of personnel and the immediate lab environment from exposure to infectious agents, and is provided by good microbiological practices, safety equipment and facility safeguards.

- Safety equipment (primary barriers) includes biological safety cabinets, enclosed containers, and other engineering controls (i.e. safety centrifuge cups) designed to remove or minimize exposure hazards to biological materials.

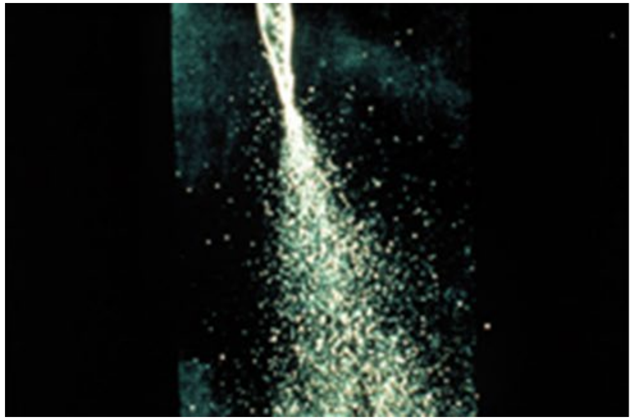
Containment

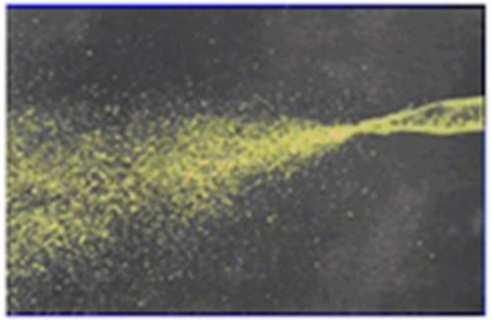
- Note: If a containment device such as a biological safety cabinet is impractical for the activity being performed, then personal protective equipment may form the primary barrier between personnel and the infectious materials.

  - Ex. Certain animal studies, agent production activities, and activities related to the maintenance, service or support of the lab facility.

Aerosol and Droplet Generating Laboratory Activities

- What lab procedures produce aerosols? Just about all of them.
  - Opening tubes, pipetting, vortexing, centrifuging, rocking/shaking cultures, sonication, tissue grinding
  - Any procedure that imparts energy into a microbial suspension
  - Animal activities, including cage changes
- The amount of aerosol generated can vary depending on technique. More efficient and careful practices will lead to a lower volume of aerosols.
- "*Aerosols are a serious hazard because they are ubiquitous in laboratory procedures, are usually undetected, and are extremely pervasive*" BMBL, 6th Ed.

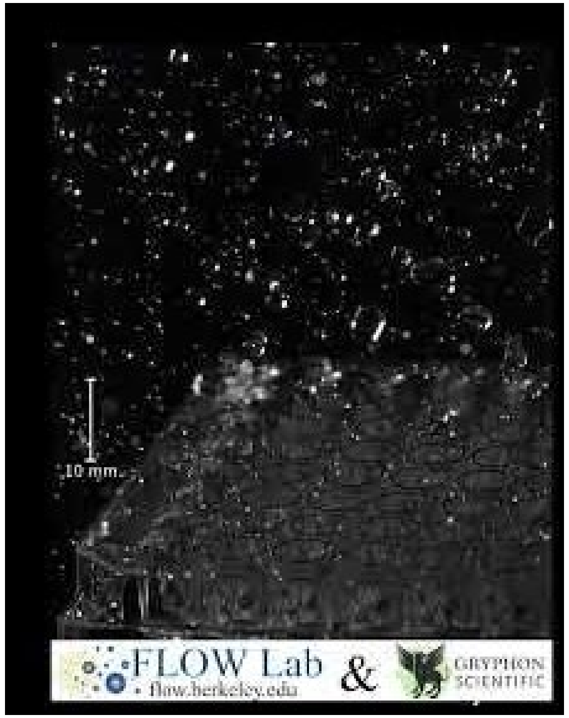




Pipette pushing out the last drop.

Aerosol and Droplet Generating Laboratory Activities

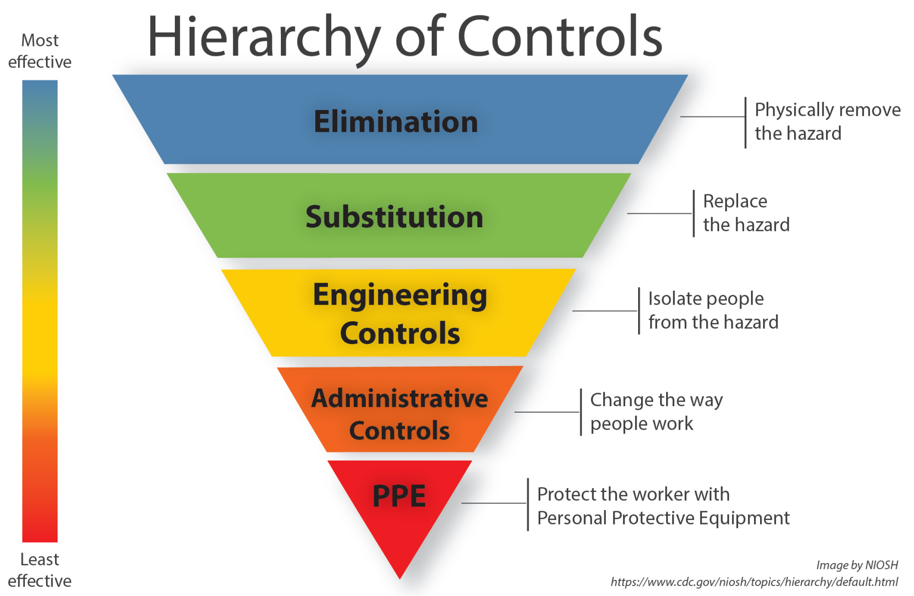
- Accidents that cause exposure via aerosol or droplet
- Dropping culture flasks, plates, petri dishes



Engineering Controls

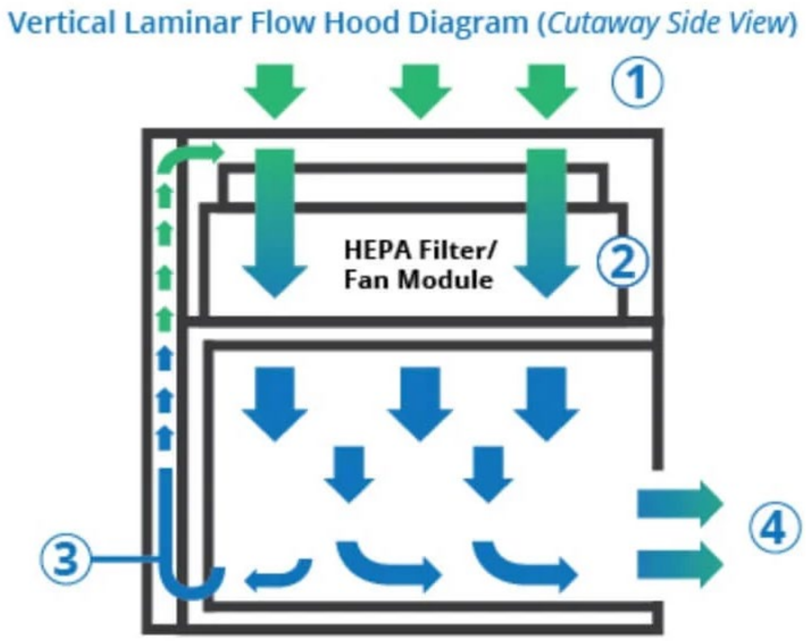
Mitigation of aerosol production can be achieved in many ways to reduce likelihood of worker and environmental exposure:

- Containment devices: biosafety cabinets and sealed centrifuge rotors and cups
  - Load and unload rotors/cups inside of BSC
  - Vortex, sonicate, grind tissue inside of BSC
- Barrier devices:
  - filtered pipette tips
  - tube opening tools (prevent glove contamination)



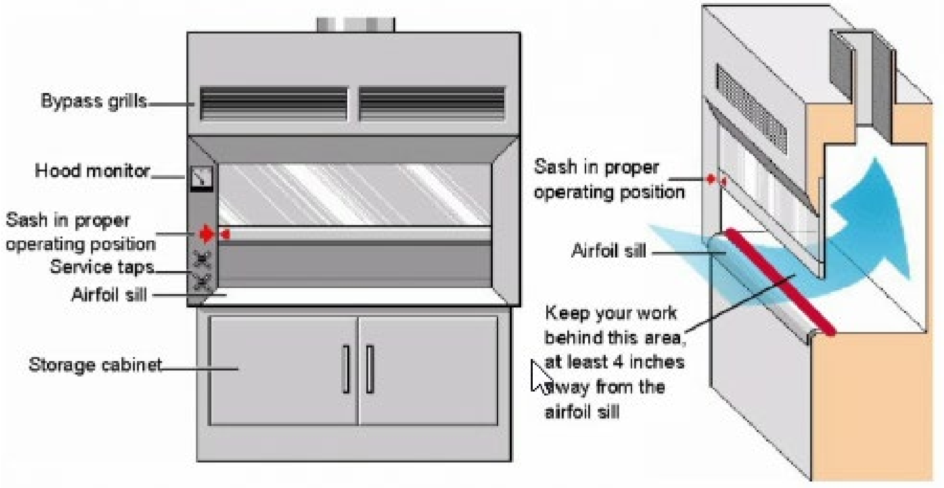
Laminar Flow Hoods (Clean Benches)

- Not to be confused with a BSC
- Laminar Flow Hoods only protect the product, not personnel protection
  - Prevents airborne contamination of the product by providing uniform HEPA-filtered air flow across the work area towards the worker
- Horizontal and Vertical laminar flow hoods
- May be used for aseptic, non-infectious work such as media plate pouring/drying



Laboratory Fume Hood

- <u>Not to be confused with a BSC</u>
- A fume hood is a ventilated enclosure in which gases, vapors and fumes are captured and removed from the work area
- Appropriate for work involving volatile chemicals and/or radionuclides
- Not appropriate for work with infectious materials
  - Offer personnel protection, but not product protection
  - No recirculation, no laminar flow over work surface
  - Exhaust not HEPA filtered



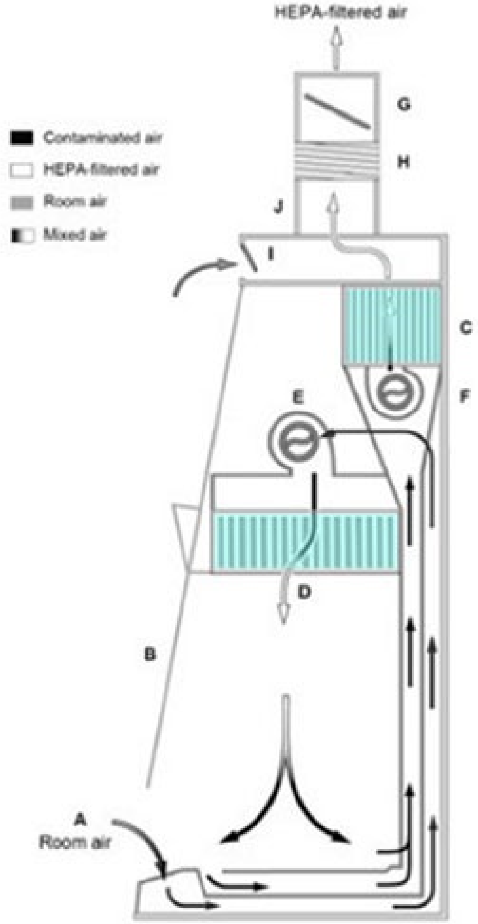
Biological Safety Cabinets

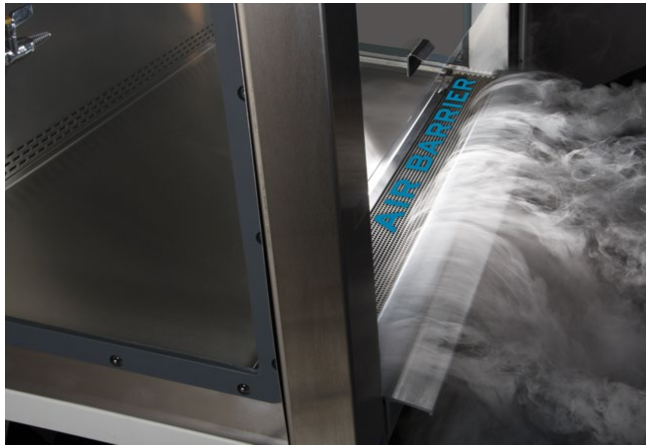
- Primary containment for work with infectious materials and protect against exposure to infectious droplets or aerosols
- If appropriately used and maintained:
  - Protects personnel from infectious agents
  - Protects environment from infectious agents
  - Protects samples/product from contamination
- Used for various research and clinical activities
- BSCs described in Appendix A of the BMBL

Biological Safety Cabinets

Directional Airflow and Air Curtain

- The restricted opening for access for the hands increases air velocity, increasing personnel protection
- HEPA filtered air is provided to the work surface, providing product protection
- Air is pulled away from the worker towards the back grate
- If the air curtain is disrupted by the potential for contaminant release is increased.





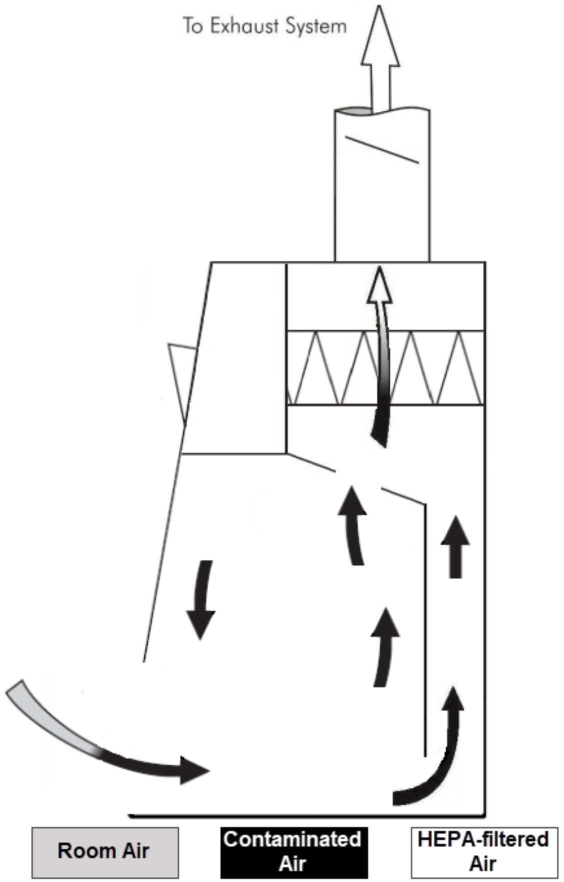
Biological Safety Cabinets

Class I	Class II	Class III
• Inward airflow protects personnel • Exhaust to outside (with/without HEPA filter)	• Personnel, Product, and Environmental protection • HEPA-filter air provided across work surface • Use for work with aerosol-transmissible microorganisms • Use also for tissue/cell culture & virology	• Personnel, Product, and Environmental protection • Totally enclosed, ventilated, air-tight • Work with BSL-3/4 agents

Biological Safety Cabinets

Class I BSC

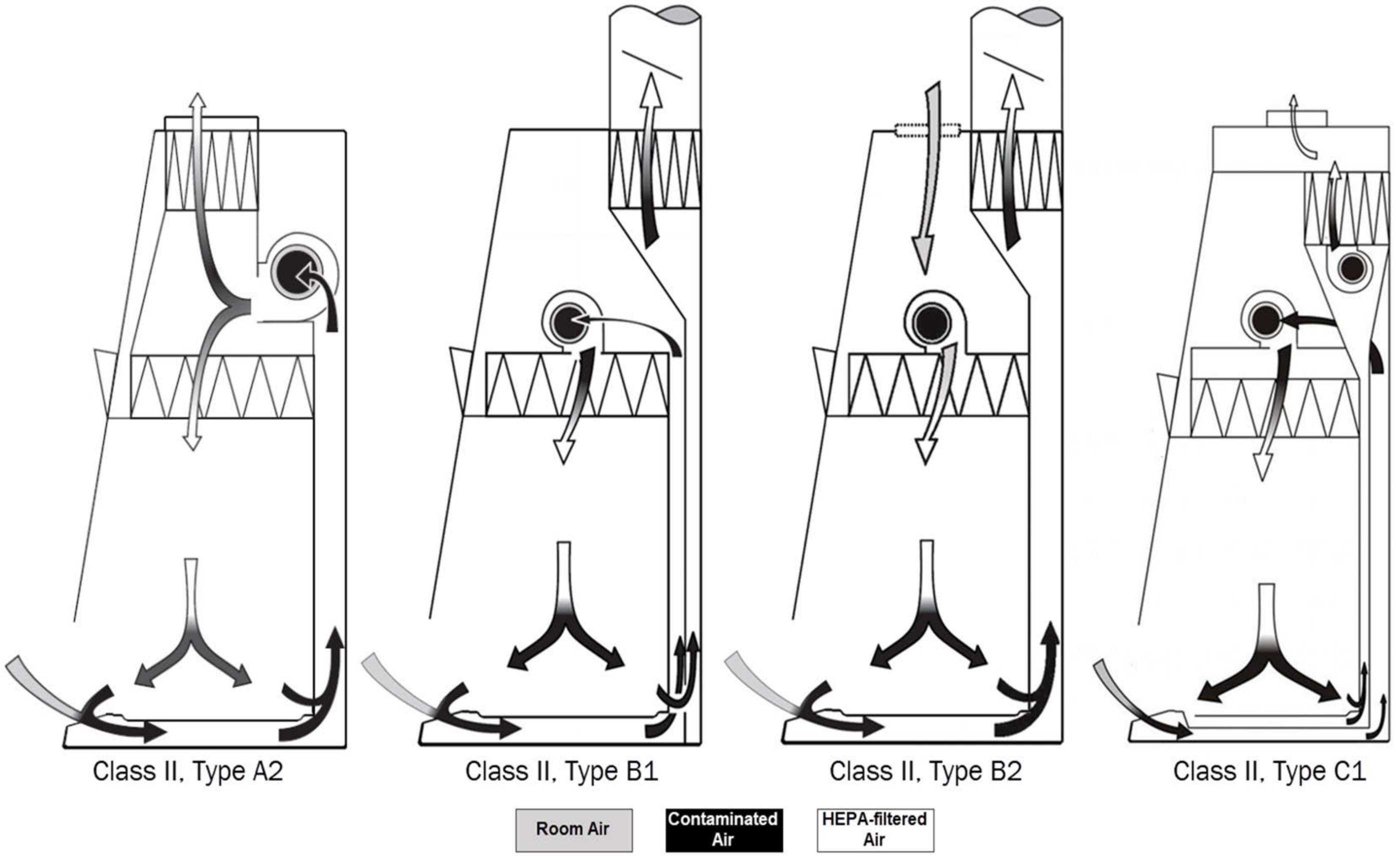
- Provides personnel and environmental protection
- Basically just a chemical fume hood with HEPA filtered exhaust
- Unfiltered air is pulled into the cabinet and across work surface
- No down flow over workspace - Does not offer product protection
- Air is pulled away from the worker
- No recirculation of air; all air is exhausted through a HEPA Filter to Building exhaust
- Common uses: where product protection is not needed such as cage dumping, culture aeration, or to enclosed equipment



Biological Safety Cabinets

Class II BSC

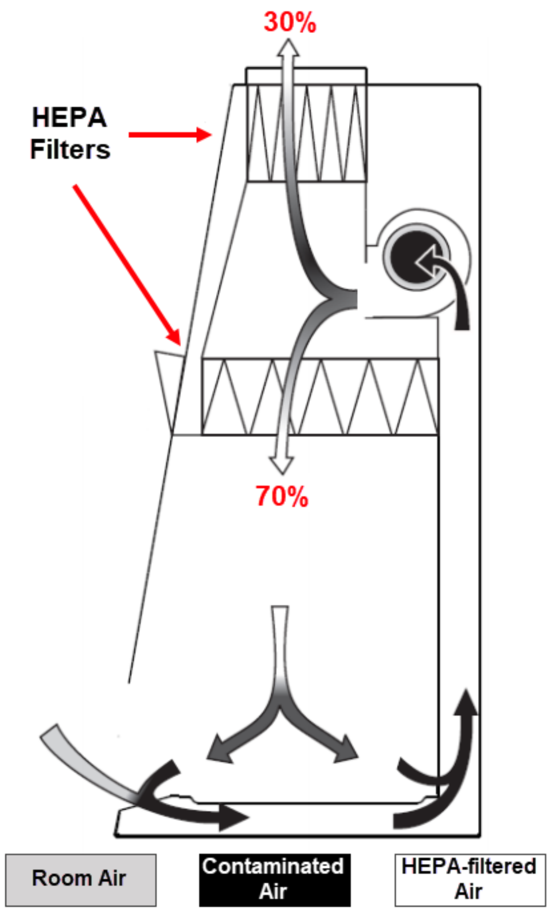
- Provides personnel, product and environmental protection
  - Air is drawn into the front grill providing personnel protection
  - HEPA-filtered "particulate-free" air provided in a downward direction to work surface which prevents cross-contamination across the work surface
  - HEPA-filtered exhaust can be recirculated to the laboratory or connected to building exhaust
- Common uses: Tissue/Cell Culture, Manipulation of Infectious Agents



Biological Safety Cabinets

Class II, Type A2

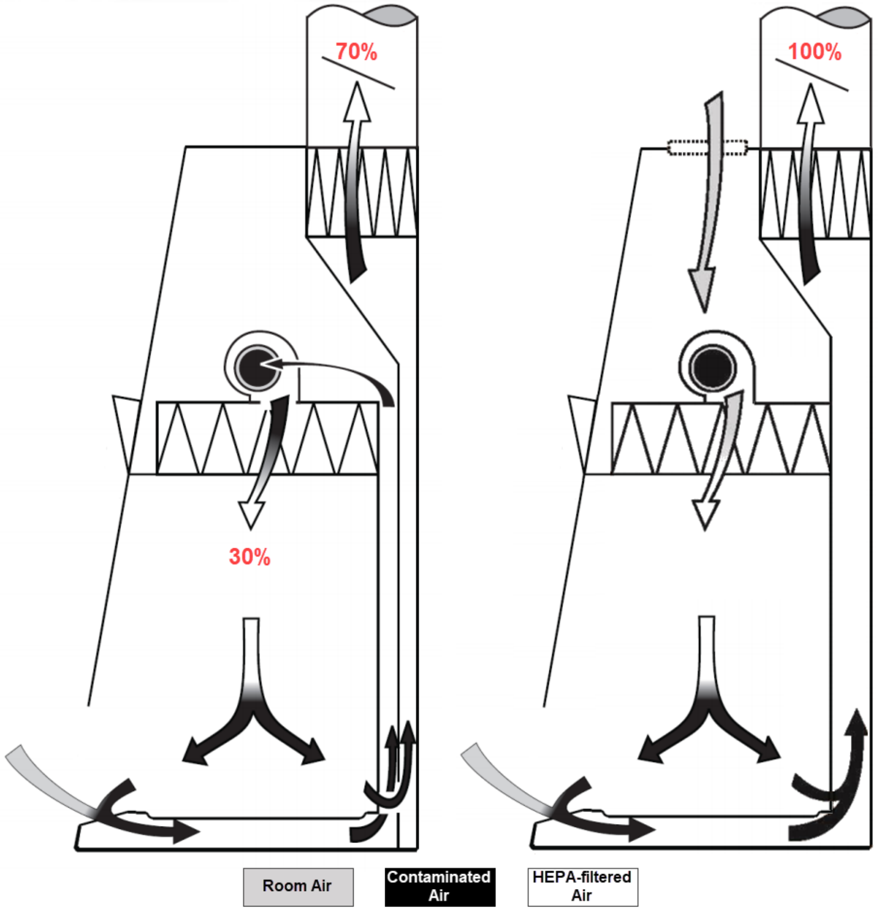
- Generally the most commonly used type of BSC
- Most (70%) of the intake air is recirculated, filtered, then used for down flow over work space
- Rest (30%) of intake air is exhausted through HEPA filter back into the lab
  - Can be ducted to building HVAC using a thimble connection, rarely done
- Because of recirculating air, A2 is not suited for work involving volatile chemicals or radionuclides
- Used at BSL-2, 3, and 4 (suit lab)



Biological Safety Cabinets

Class II, Types B1 and B2

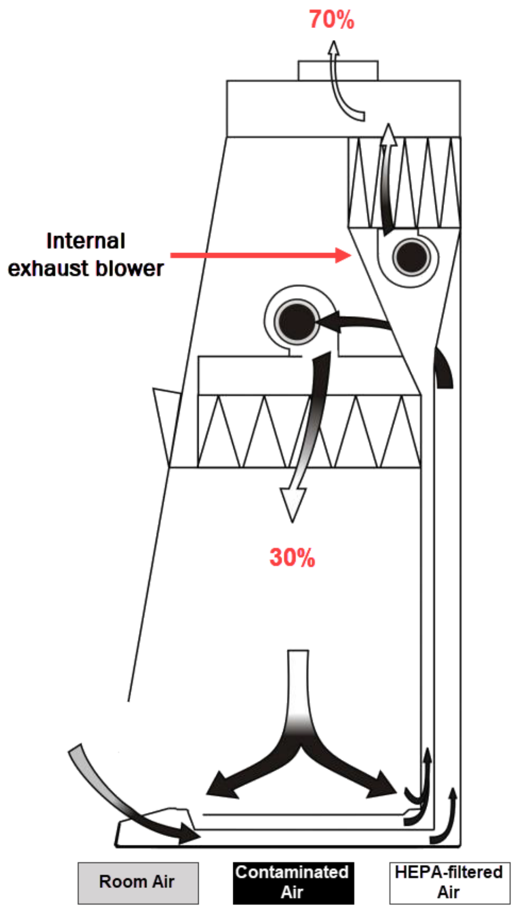
- Type B BSCs are exhausted to building HVAC rather than into the lab
  - Have to be hard ducted into building exhaust
- Allow for more freedom in what you can work with
  - Volatile chemicals or radionuclides
- Susceptible to problems resulting from any variation in HVAC air flow
- Used at BSL-2, 3, and 4 (suit lab)
- B1/B2 main difference: recirculation (B1) or not (B2)



Biological Safety Cabinets

Class II, Type CI

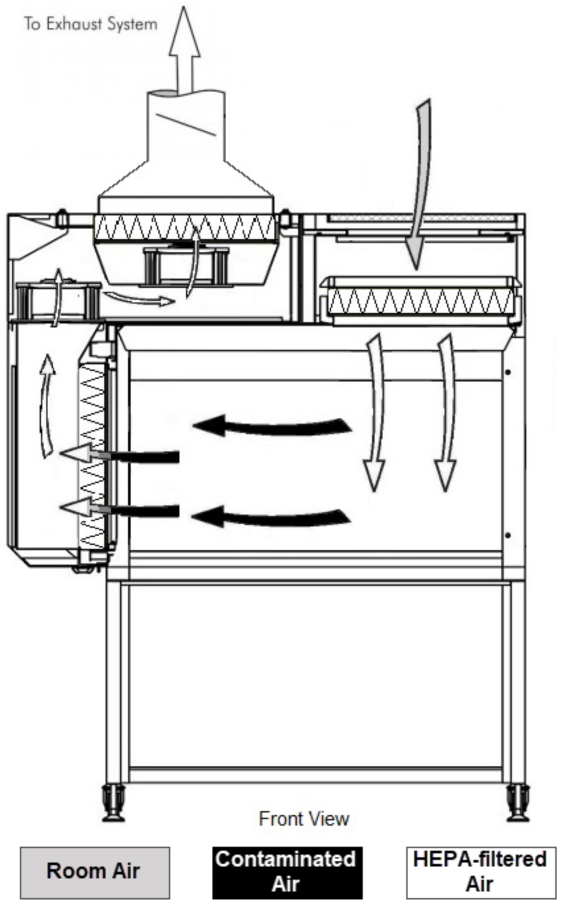
- A more recently introduced type of BSC
- Can operate similarly to an A2 or B1 cabinet
- Exhaust into lab if not ducted
- Exhaust into building HVAC if ducted
  - If ducted to building HVAC: Internal exhaust blower allows operation for ~5 minutes after building HVAC failure
- Can work with small amounts of volatile chemicals



Biological Safety Cabinets

Class III "Glove Box"

- Gas-tight construction, manipulations are done using heavy-duty gloves attached to the cabinet
- Supply and exhaust air are HEPA filtered
- Exhaust air is either double-HEPA filtered or HEPA filtered and then incinerated
- Samples are placed or removed via a pass-through dunk tank or autoclave
- Used at BSL-3 and 4 (cabinet lab)

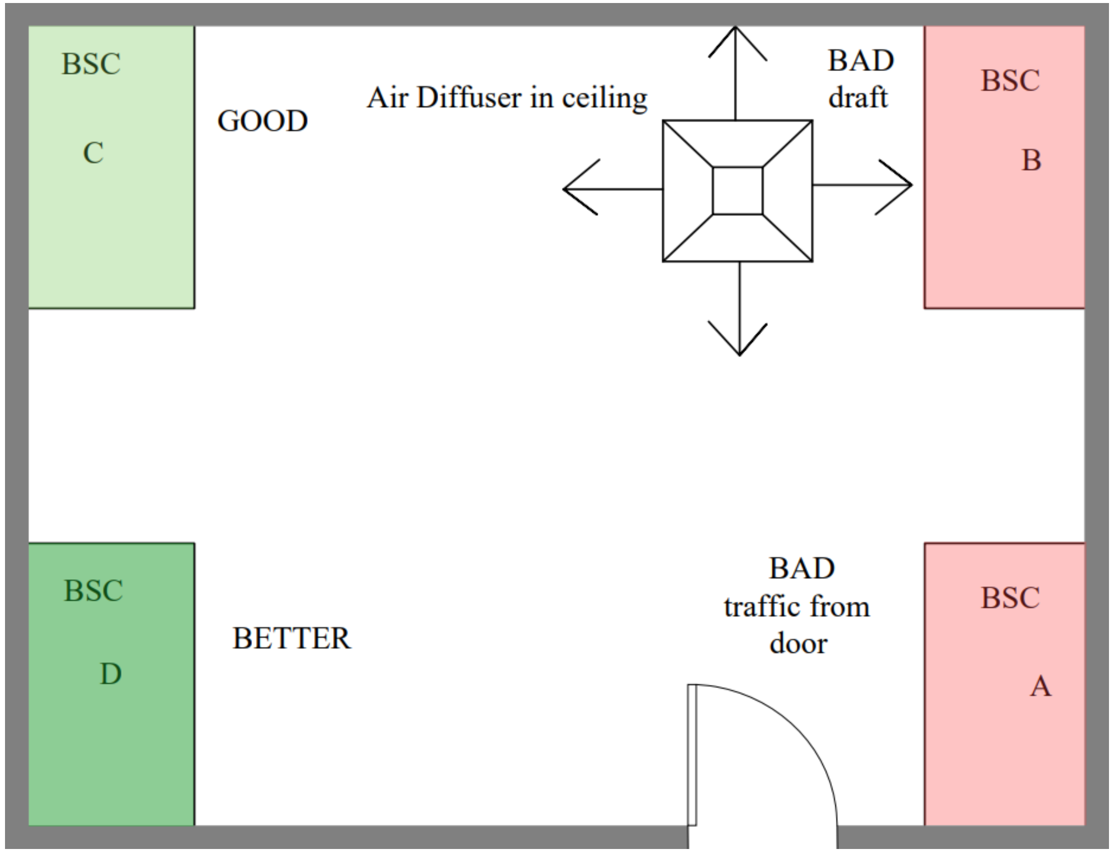


Biological Safety Cabinet Placement

When installing a BSC in a lab, it is important to choose a location that will prevent air flow disturbances

Areas to avoid:

- High traffic areas/near doorways
- Near HVAC vents



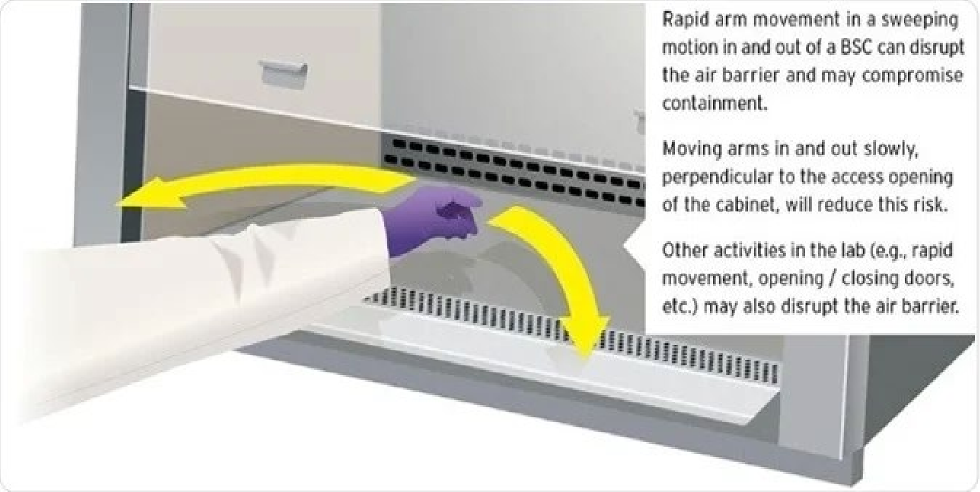
Biological Safety Cabinet Operation

Before use:

- Check that BSC is ON and air is flowing
  - If turning it on: allow at least 5 minutes before beginning work – filter's electrostatic charge builds from airflow
- Check that certification is current – within a calendar year
- Check that the pressure gauge reading is at or slightly above the certification pressure
  - May be a digital display rather than a Magnehelic
  - Lower pressure indicates a tear in the filter
  - Higher pressure could indicate obstruction of the filter
- Disinfect inside surfaces before placing supplies/samples in the BSC, including work surface, sides, back, and inside of the window
  - If using bleach or other corrosive disinfectant, follow with ethanol
- Ensure UV light is OFF when in use

Biological Safety Cabinet Best Practices

- Minimize potential airflow disturbances into the cabinet.
- Activities that disturb airflow are:
  - Crossing hands/arms
  - Moving in/out of the cabinet, side to side
  - People walking close by, opening the lab door
- Do not block grilles at the front and back of the cabinet
- Disinfect or change gloves before entering/exiting the cabinet
- Organize workspace such that:
  - There are designated “clean” and “dirty” zones
  - Crossing your hands or arms is minimized
- Work at least 4” inside the cabinet
- Manipulation of materials should be delayed for approximately 1 minute after placing hands/arms inside the cabinet



Biological Safety Cabinet Best Practices

- A risk assessment by the IBC committee must be conducted if two personnel are working within the BSC simultaneously for different protocols/agents
- BSCs are designed for 24-hour / day operation
- Biohazard waste (small bag/small sharps container) should be inside the BSC during work, not outside of the BSC
- Large items within the BSC will disrupt the directional airflow

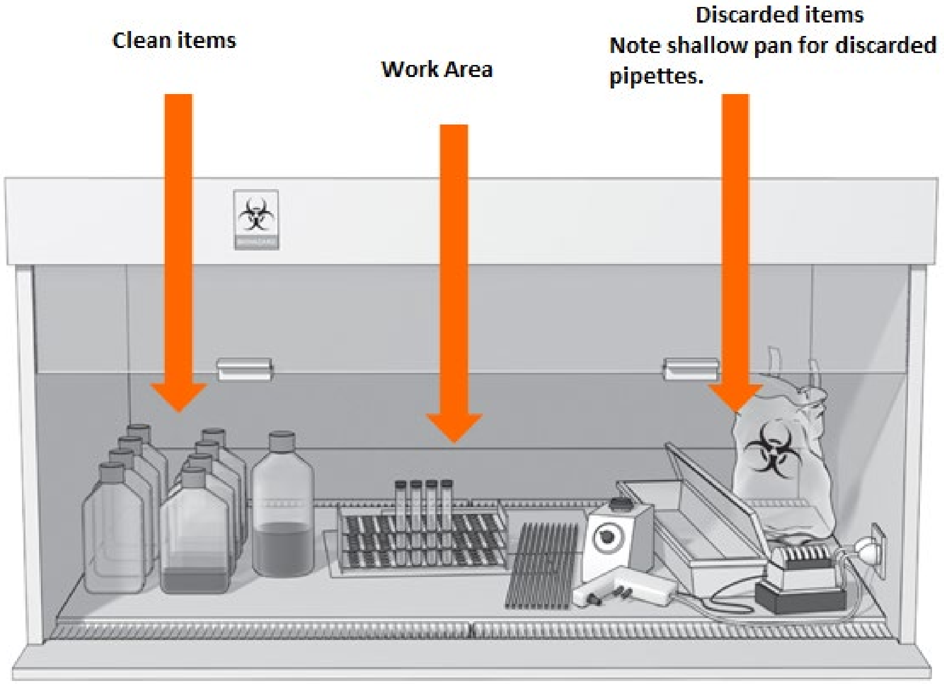
Biological Safety Cabinet Best Practices

- Clean to Dirty: Clean cultures (left) can be inoculated (center); contaminated pipettes can be discarded in the shallow pan and other contaminated materials can be places in the biohazard bag (right).
- Note: This arrangement is reversed for left-handed persons.

- Plastic-backed, adsorbent toweling can be placed on the work surface (not blocking grates)

- Only materials and equipment required for the immediate work should be placed in the BSC

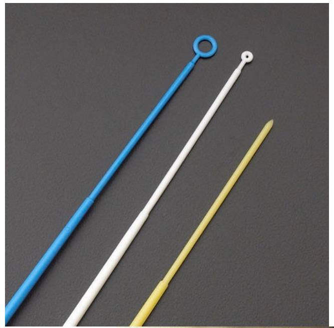
- Extra supplies should be stored outside of the BSC



Biological Safety Cabinet Best Practices

Open Flames (i.e., Bunsen burners) are **not allowed in the BSC**

- BSCs already have features to protect the samples being worked with there is no need for flame sterilization
- Heat rises, causing turbulence that interferes with the air flow over work surface
- Potential for damaging the HEPA filter or causing a build-up of gas and explosion risk
- Alternatives to an open flame include:
    - Bacti-Cinerator and similar devices use IR contained within the tube to sterilize loops safely
    - Disposable inoculating loops



Biological Safety Cabinet Best Practices

When work is complete:

- Place all waste in waste container(s) within the BSC
    - Close waste container, disinfect and remove from BSC to appropriate laboratory waste container.
    - If items are left in a tray with disinfectant, the BSC should remain ON
- Remove all materials used during the work from the BSC
    - Surface disinfect items before removal from BSC
- Disinfect inside surfaces of cabinet – same as before starting
- If the cabinet motor is left on, do not close the sash completely, could burn out the motor

Biological Safety Cabinet Best Practices

Disinfect the BSC :

- Keep BSC ON
- Select the appropriate disinfectant for the agent
- Ensure appropriate contact on surfaces and let the disinfectant stay on the surface for the appropriate contact time
- Work surface, cabinet sides and back and interior of window

- Disinfect before and after working in the BSC
  - Before: protects your experiments/products
  - After: protects you/the environment

Biological Safety Cabinet Best Practices

UV Lights:
- Can’t disinfect where there are shadows
- Dust on the bulb can reduce effectiveness
- Lights have limited lifespan
- Should not be the primary method of disinfection before/after working in the BSC
- If using UV, do not work inside the BSC while the light is turned on

Biological Safety Cabinet Certification

The operational integrity of Class II BSC must be tested and certified at least annually in addition to:

- Upon installation of the BSC (new or reused)
- Any time the BSC is moved
- After any repairs or filter changes

NSF/ANSI 49 (current version released in 2022): Biosafety Cabinetry – Design, Construction, Performance, And Field Certification

Must be approved vendor with accredited field certifiers

After certification the vendor will place a certification sticker on the BSC

Biological Safety Cabinet Certification

During a certification the following tests are required:

- **Downflow velocity profile test:** Measures the velocity of air moving through the cabinet
- **Inflow velocity test:** Measures velocity of air moving through unit opening (face velocity)
- **HEPA filter leak test:** Ensure integrity of downflow and exhaust HEPAs, filter housings, and filter mounting frames
- **Site installation assessment tests:** Airflow and sash alarms, Interlocks and exhaust system performance/canopy connection
- **Airflow smoke patterns test:** Ensures inward airflow along sash opening, downward airflow within the work area with no dead spots/refluxing, no ambient passing on or over the work surface, and no escape to the outside of the cabinet at the sides and top of the window
- **Cabinet integrity tests (Type A1 only):** Determines if exterior of all plenums, welds, gaskets, and plenum penetrations/seals are free of leaks

HEPA Filters

“High efficiency particular air’’ filter

- Traps particulates only; chemicals, fumes, vapors pass through
- Traps particulates at highest efficiency at 0.3µ particle size
- Typically constructed of paper-thin sheets of borosilicate medium, pleated to increase surface area, and affixed to a frame.
- Aluminum or plastic separators are often added for stability.

  - In a Biosafety Cabinet
  - In Facilities Air Supply (ISO Clean Rooms) or Exhaust (BSL3/4)
  - Small filters to protect facility vacuum lines
  - Designed into equipment

HEPA Filters

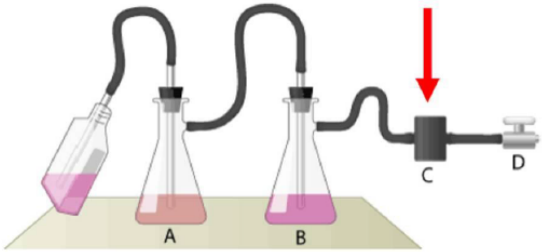
"High efficiency particular air'' filter

- Inline HEPA Filter
  - Used with liquid biohazards collection into flasks
  - To protect vacuum lines

Protection of Vacuum Systems Used in Tissue Culture Work

CDC/NIH Guidelines for Biosafety Level 2 (BSL-2 laboratories) state that vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may also be required.

Aspirator bottles or suction flasks (A) containing appropriate disinfectant should be connected to overflow collection flasks (B) containing appropriate disinfectant, and to an in-line HEPA filter (C) or equivalent filter before the vacuum line (D). This combination will provide protection to the central building vacuum system or vacuum pump, as well as to the personnel who service this equipment. Inactivation of aspirated materials can be accomplished by placing sufficient chemical decontamination solution into the flask to kill the microorganisms as they are collected.
Once inactivation occurs, liquid materials can be disposed of as non-infectious waste.
Flasks should be emptied when no more than ½ full. Flasks should be kept inside secondary containment.



CDC/NIH: Biosafety in Microbiological and Biomedical Laboratories (BMBL) *6th Edition 2020*

Use an in-line hydrophobic, filter made of PTFE with a 0.3µm particle retention.

Filters are available from Fisher Scientific and other manufacturers

Name	Properties	Dimensions	Part Number	
Vacushield Vent Device, Pall Life Sciences	Hydrophobic PTFE membrane filter, 0.3 µm particle retention, autoclavable	Stepped hose barbs 6.4-12.7 mm diameter, internal taper accepts standard mule luer	Pall 4402	
Whatman HEPA –Vent Filter	Mildly hydrophobic, 0.3 µm particle retention, autoclavable	Inlet/outlet 1/4 to 3/8 inch stepped barb	Whatman 6723-5000	
Millipore Millex Vacuum Line Protection	Hydrophobic PTFE membrane filter, 0.2 µm particle retention, autoclavable	Variety of inlet/outlet combinations including stepped hose barb and 1/8 NPTM	Millipore SLFG75010	

HEPA Filters

- May be incorporated into equipment that is aerosol-generating
- Flow Cytometry / Cell Sorter
- Centrifuges
- Each manufacturer has a recommended containment option, usually an attachment or an enclosure, that can be utilized for the confinement of biohazards.

Centrifuges

- Only use centrifuges that have either sealed rotors or sealed cups
- Check the seals on these regularly to ensure they are not drying/cracking
- Inspect tubes for cracks before and after a run
- Balance tubes in the rotor/cups
- Sealed Rotors – Rotors should be removed from the centrifuge and **loaded/unloaded inside the BSC**
- Sealed Cups– Cups should be removed from the centrifuge and **loaded/unloaded inside the BSC**
- Allow centrifuge to sit at least 5 minutes after completing a run
    - Allows aerosols to settle if a tube breaks or leaks
- May have option for HEPA filter as part of design

Barrier Devices

In addition, tools can be used as barrier devices to minimize aerosol generation and /or ensure containment

- filtered pipette tips
- tube opening tools (prevent glove contamination)

References

- NSF/ANSI 49-2022: Biosafety Cabinetry: Design, Construction, Performance, And Field Certification
- U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, & National Institutes of Health. (2007). Biosafety in microbiological and biomedical laboratories (6th ed.). Washington, DC: U.S. Government Printing Office. Available at:https://www.cdc.gov/labs/BMBL.html

Questions

If you have any questions or concerns, please contact the Biological Safety Program at 713-500-8170

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