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8.1 Overview

A biological safety cabinet is used as a primary barrier against exposure to infectious agents. A PRIMARY BARRIER SUCH AS A BIOLOGICAL SAFETY CABINET MERELY COMPLEMENTS CAREFUL WORK PRACTICES.

Biological safety cabinets contain High Efficiency Particulate Air (HEPA) filters, which have 99.97% to 99.99% efficiency for 0.3 micron-sized particles. These cabinets operate with laminar air flow, which is the movement of air with uniform velocity in one direction along parallel flow lines, either horizontally or vertically.

8.2 Primary Containment: Biological Safety Cabinets

Biological Safety Cabinets (BSCs) are among the most effective and the most commonly used primary containment devices in laboratories working with infectious agents. The three general types available (Class I, II, III) have performance characteristics and applications, which are described in this appendix. Properly maintained Class I and II BSCs, when used in conjunction with good microbiological techniques, provide an effective containment system for safe manipulation of moderate and high-risk microorganisms (Biosafety Level 2 and 3 agents). Both Class I and II BSCs have inward face velocities (75-100 linear feet per minute) that provide comparable levels of containment to protect laboratory workers and the immediate environment from infectious aerosols generated within the cabinet. Class II BSCs also protect the research material itself through high-efficiency particulate air filtration (HEPA filtration) of the air flow down across the work surface (vertical laminar flow). Class III cabinets offer the maximum protection to laboratory personnel, the community, and the environment because all hazardous materials are contained in a totally enclosed, ventilated cabinet.

8.3 Class I

The Class I Biological Safety Cabinet (Fig. 1) is a negative-pressure, ventilated cabinet usually operated with an open front and a minimum face velocity at the work opening of at least 75 linear feet per minute (lfpm). All of the air from the cabinet is exhausted through a HEPA filter either into the laboratory or to the outside. The Class I BSC is designed for general microbiological research with low and moderate-risk agents, and is useful for containment of mixers, blenders, and other equipment. These cabinets are not appropriate for handling research materials that are vulnerable to airborne contamination, since the inward flow of unfiltered air from the laboratory can carry microbial contaminants into the cabinet.

The Class I BSC can also be used with an installed front closure panel without gloves, which will increase the inward flow velocity to approximately 150 lfpm. If such equipped cabinets are ducted to the outside exhaust, they may be used for toxic or radiolabelled materials used as an adjunct to microbiological research. Additionally, arm-length rubber gloves may be attached to the front panel with an inlet air pressure release for further protection. In this configuration, it is necessary to install a make-up air inlet fitted with a HEPA filter in the cabinet.

8.4 Class II
The Class II Biological Safety Cabinet (Fig. 2.a) is designed with inward air flow at a velocity to protect personnel (75-100 lpfm), HEPA-filtered downward vertical laminar airflow for product protection, and HEPA-filtered exhaust air for environmental protection. Design, construction, and performance standards for Class II BSCs, as well as a list of products that meet these standards, have been developed by and are available from the National Sanitation Foundation International,(2)Ann Arbor, Michigan. Utilization of this standard and list should be the first step in selection and procurement of a Class II BSC.

Class II BSCs are classified into two types (A and B) based on construction, air flow velocities and patterns, and exhaust systems. Basically, Type A1 and A2 cabinets are suitable for microbiological research in the absence or very small amounts of volatile or toxic chemicals and radionuclides, since air is recirculated within the cabinet. Type A cabinets may be exhausted into the laboratory or to the outdoors via a "thimble" connection to the building exhaust system. Type B cabinets are further sub-typed into types B1 and B2. A comparison of the design features and applications are presented in Figures 2b, 2c, and 2d, respectively. Type B cabinets are hard-ducted to the building exhaust system and contain negative pressure plena. These features, plus a face velocity of 100 lpfm, allow work to be done with toxic chemicals or radionuclides.

It is imperative that Class I and II biological safety cabinets be tested and certified in situ at the time of installation within the laboratory, at any time the BSC is moved, and at least annually thereafter. Certification at locations other than the final site may attest to the performance capability of the individual cabinet or model but does not supersede the critical certification prior to use in the laboratory.

As with any other piece of laboratory equipment, personnel must be trained in the proper use of the biological safety cabinets. Of particular note are activities that may disrupt the inward directional airflow. Repeated insertion and withdrawal of the workers' arms into and out of the work chamber, opening and closing doors to the laboratory or isolation cubicle, improper placement or operation of materials or equipment within the work chamber, or brisk walking past the BSC while it is in use have been demonstrated to cause the escape of aerosolized particles from within the cabinet. Class I and II cabinets should be located away from traffic patterns and doors. Air flow from fans, room air supply louvers and other air moving devices can disrupt the airflow pattern at the face of the cabinet. Strict adherence to recommended practices for the use of BSCs and their proper placement in the laboratory are as important in attaining the maximum containment capability of the equipment as is the mechanical performance of the equipment itself.

8.5 Class III

The Class III Biological Safety Cabinet (Fig. 3) is a totally enclosed, ventilated cabinet of gas-tight construction and offers the highest degree of personnel and environmental protection from infectious aerosols, as well as protection of research materials from microbiological contaminants. Class III cabinets are most suitable for work with hazardous agents that require Biosafety Level 3 or 4 containment.

All operations in the work area of the cabinet are performed through attached arm length rubber gloves or half-suits. The Class III cabinet is operated under negative pressure. Supply air is HEPA-filtered and the cabinet exhaust air is filtered through two HEPA filters in series, or HEPA filtration followed by incineration, before discharge outside of the facility.
All equipment required by the laboratory activity, such as incubators, refrigerators, and centrifuges, must be an integral part of the cabinet system. The Class III cabinet must be connected to a double-doored autoclave and/or chemical dunk tank used to sterilize or disinfect all materials exiting the cabinet, and to allow supplies to enter the cabinet. Several Class III cabinets are therefore typically set up as an interconnected system.

8.6 Positive-Pressure Personnel Suit

Personnel protection equivalent to that provided by Class III cabinets can also be obtained with the use of a one-piece, ventilated suit worn by the laboratory worker when working with Biosafety Level 4 agents in a "suit area" and using Class I or II BSCs. The personnel suit is maintained under positive pressure with a life-support system to prevent leakage into the suit. In this containment system, the worker is isolated from the work materials.

The personnel suit area must be essentially equivalent to a large Class III cabinet. The area is entered through an air-lock fitted with airtight doors. A chemical shower is provided as a "dunk tank" to decontaminate the surfaces of the suit as the worker leaves the area. The exhaust air from the suit area is filtered through two HEPA filter units installed in series. The entire area must be under negative pressure.

As with Class III BSCs, the gloves of the personnel suit are the most vulnerable component of the system, as they are subject to punctures by sharps or animal bites.

8.7 Other Devices

Other Devices: Horizontal laminar flow "clean benches" are used in clinical, pharmaceutical, and laboratory facilities strictly for product protection. This equipment must never be used for handling toxic, infectious, radioactive, or sensitizing materials, since the worker sits in the immediate downstream exhaust from the "clean bench." Vertical laminar flow benches may be useful for certain manipulations of clean materials (e.g., pouring agar plats) but should not be used when working with infectious materials.

References


National Sanitation Foundation Standard 49. 2007.
Class II (Laminar Flow) Biohazard Cabinetry. Ann Arbor, Michigan.
### Table 8.8 - Comparison of Biological Safety Cabinets

<table>
<thead>
<tr>
<th>Type</th>
<th>Face velocity (lfpm)</th>
<th>Airflow Pattern</th>
<th>Radionuclides/Toxic Chemicals</th>
<th>Biosafety Level(s)</th>
<th>Product Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I* open front</td>
<td>75</td>
<td>In at front; rear and top through HEPA filter</td>
<td>No</td>
<td>2,3</td>
<td>No</td>
</tr>
<tr>
<td>Class II Type A1</td>
<td>75</td>
<td>70% recirculated through HEPA; exhaust through HEPA</td>
<td>No</td>
<td>2,3</td>
<td>Yes</td>
</tr>
<tr>
<td>Type A2</td>
<td>100</td>
<td>Same as IIA1, but plena under negative pressure to room and exhaust air is ducted</td>
<td>Yes</td>
<td>2,3</td>
<td>Yes</td>
</tr>
<tr>
<td>Type B1</td>
<td>100</td>
<td>30% recirculated through HEPA; exhaust via HEPA and hard ducted</td>
<td>Yes (Low levels/volatility)</td>
<td>2,3</td>
<td>Yes</td>
</tr>
<tr>
<td>Type B2</td>
<td>100</td>
<td>No recirculation; total exhaust via HEPA and hard ducted</td>
<td>Yes</td>
<td>2,3</td>
<td>Yes</td>
</tr>
<tr>
<td>Class III</td>
<td>NA</td>
<td>Supply air inlets and exhaust through 2 HEPA filters</td>
<td>Yes</td>
<td>3,4</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*Glove panels may be added and will increase face velocity to 150 lfpm; gloves may be added with an inlet air pressure release that will allow work with chemicals/radionuclides.
8.9 **Class I Biological Safety Cabinet**

Figure 1.
Class I Biological Safety Cabinet.

A. front opening, B. sash, C. exhaust HEPA filter, D. exhaust plenum
8.10  **Class II, Type A1 Biological Safety Cabinet**

![Diagram of Class II, Type A1 Biological Safety Cabinet](image)

- A. front opening, B. sash, C. exhaust HEPA filter, D. rear plenum, E. supply HEPA filter, F. blower

8.11  **Table Top Model of a class II, type A2 Biological Safety Cabinet**

![Diagram of Table Top Model](image)

- A. front opening, B. sash, C. exhaust HEPA filter, D. supply HEPA filter, E. positive pressure plenum, F. negative pressure plenum.
8.12 Class II, Type B1 Biological Safety Cabinet (Classic Design)

Figure 2c.

Connection to building exhaust system required.

A. front opening, B. sash, C. exhaust HEPA filter, D. supply HEPA filter, E. negative pressure exhaust plenum, F. blower, G. additional HEPA filter for supply air. Note: The cabinet exhaust needs to be connected to the building exhaust system.

8.13 Class II, Type B2 Biological Safety Cabinet

Figure 2d.

Connection to building exhaust system required.
A. front opening, B. sash, C. exhaust HEPA filter, D. supply HEPA filter, E. negative pressure exhaust plenum, F. filter screen. Note: The carbon filter in the building exhaust system is not shown. The cabinet exhaust needs to be connected in the building exhaust system.

### 8.14 Class III Biological Safety Cabinet

**Figure 3**

Connection to building exhaust system required.

A. glove ports, with O-ring for attaching arm-length gloves to cabinet. B. sash, C. exhaust HEPA filter, D. supply HEPA filter, E. double-ended autoclave or pass-through box. Note: A chemical tank may be installed which would be located beneath the work surface of the BSC with access from above. The cabinet exhaust needs to be connected to the building exhaust system.

### 8.15 Certification

Never use a biological safety cabinet unless it has been certified to meet minimum safety specifications (e.g., NIH-03-112 or NSF Standard No. 49). Every biological safety cabinet will be certified by qualified personnel at the following times:

- when newly installed
- after filter replacement
- after repairs
- after the cabinet has been moved
- annually

EHS maintains an inventory of biological safety cabinets and schedules the annual certification inspections. EHS-B&C provides free certification of HEPA filtered equipment. Call extension 21781 for more information.
8.16 SAFETY RULES FOR USE OF CLASS I AND II CABINETS

8.16.1. Understanding the monitors

Before any work is performed in a biosafety cabinet it is very important to verify the air flow monitors to determine if work can or cannot be safely performed.

There are several aspects to understand when looking at those monitors:
- Will both you and the sample be protected from one another?
- Will the biosafety cabinet protect you from the biological hazards that are inside it?
- Will the samples in the biosafety cabinet be protected from the outside environment?

If the biosafety cabinet is in alarm, you will be protected from the biohazards as long as the cabinet is in negative pressure, meaning that it is pulling air inside the biosafety cabinet; on the other hand, your samples are at risk of contamination. If the biosafety cabinet indicates a positive pressure then you are at risk of being exposed as the air inside the cabinet is being pulled out into the room; if this should happen, close the sash immediately, notify co-workers present to leave the room, post DO NOT ENTER BIOHazard Exposure signs on the door and notify your supervisor immediately.

If the alarm on the biosafety cabinet goes on this could be an indication of pressure problems in the room you need to check the room pressure ASAP, as the room pressure might indicate that you have to leave immediately.

There are different types of monitor displays depending on the biosafety cabinet manufacturer.

Some will be on a digital readout with green, yellow and red lights.
If the green light is between -1 and +1 then you can safely work in the biosafety cabinet - both you and the samples are protected.
If the red light is between -1 and -2 then an alarm should sound stating that there are risks in working in the biosafety cabinet. You should not work in the biosafety cabinet if this is the case. If you had started working in the biosafety cabinet, then you are protected, but your samples are not protected - you should stop working as soon as possible and contact your supervisor.
If the red light is between +1 and +2 then you should not start working in the biosafety cabinet. If you had started working then immediately stop, close the sash, evacuate the room and contact your supervisor.

Numeric readout
If your biosafety cabinet has a numeric readout, check the pressure indicated on the certification label - this number will tell you the pressure the cabinet was operating with the last time it was certified. As time passes, the number in the numeric readout (Magnahelic) will gradually increase as the HEPA filters load. This is normal. However, if you see a major drop in the magnahelic reading, this could indicate a leak in a seal or a hole in the HEPA. You should NOT work in the cabinet in this case – contact your supervisor immediately.
8.16.2 Starting the job

Before starting work verify the proper functioning of the biosafety cabinet. Verify that the drain valve is closed (if present on the BSC model). Disinfect interior surfaces using alcohol, Cavicide, or another appropriate disinfectant.

- Everything needed for the complete procedure shall be placed in the cabinet before starting work. All materials going into the cabinet should be surface wiped/sprayed with disinfectant. Nothing shall pass in or out through the air barrier until the procedure is completed. Avoid overloading the work area, and thereby compromising the efficacy of the BSC.
- Work supplies are best arranged to segregate clean from dirty materials.
- Wait five minutes after all materials have been placed in the BSC before beginning work. This will enable the BSC to purge airborne contaminants from the work area.
- Adjust chair height so that the sash bottom is at armpit level. Work as far to the back of the BSC work space as possible.
- Place a waste container containing a 10% bleach solution for the contaminated material (pipettes, tubes, plates, tips....) as well as a waste container for the papers waste in the BSC before starting work. Replace container if they get filled.
- Avoid using toxic, explosive, flammable, or radioactive substances in the BSC unless a safety professional has approved the procedure.

8.16.3 Completion of a Job

When finished using a biosafety cabinet, make sure that:

- all equipment which has been placed in the BSC will be surface decontaminated prior to removal from the cabinet
- waste containers are covered
- the cabinet is allowed to operate for five minutes with no activity in order to purge airborne contaminants from the work area
- interior work surfaces are decontaminated
- you thoroughly wash your hands and arms with warm, soapy water

8.16.4 Removal of material from the biosafety cabinet

Materials that need to be removed from the biosafety cabinet need to be decontaminated prior to removal.

- Pipette wrappers, paper towels, etc should be sprayed with disinfectant before being moved to the trash.
- Biological materials that need to go into an incubating chamber should be wiped down and double contained.
- Biological materials that need centrifugation should be placed into a sealed centrifugation container (e.g., sealed safety cups or sealed rotor) which is disinfected prior to leaving the biosafety cabinet.
Biohazard waste should be placed into a secondary container that will be disinfected and autoclaved.

8.16.5 Biohazardous and rDNA Spills in the Cabinet

In case a biohazardous or rDNA spill occurs inside the cabinet:

- Decontamination steps should be taken while the cabinet is operating to prevent the escape of contaminants. Change gloves (lab coat if contaminated) and inform lab co-worker of spill, as they may want to leave (but evacuation is not necessary).
- Cover spill with absorbent material and pour on disinfectant, let soak for 15-30 minutes, then remove absorbent material (double bag for disposal) and call P.I.
- Spray or wipe walls, work surface, and all apparatus that is affected with an appropriate disinfectant. (Make sure to wear gloves while doing this.)
- Empty biosafety cabinet; decontaminate all materials as they are removed; clean under tray
- If a drain system is involved, consult the BSC manufacturer’s specific instructions regarding decontamination. Bag all cleaning materials.
- Change PPE.

After a spill is decontaminated, the cabinet shall be thoroughly cleaned and dried. Residual materials can support the growth and multiplication of microorganisms, and can jeopardize the product protection normally provided by biological safety cabinets.

8.16.6 Remember

- The biological safety cabinet is not a substitute for good laboratory practice.
- Aerosols can escape.
- The airflow is disrupted by:
  - rapid movement of hands or arms,
  - opening doors to the room,
  - persons walking past the cabinet.
- Decontaminate the cabinet before and after each use.

8.16.7 Do's and Don'ts of the biosafety cabinet

Do:
- Work with any infectious or potentially infectious agents
- Keeps work area free of unnecessary clutter including equipment and supplies. This may result in a loss of proper air flow and a high risk of incidence.
- Organize your work time to not have to rush
- Keep amount of work to be done realistic within the time frame
- ALWAYS surface decontaminate all surfaces and material coming out of the biosafety cabinet.
- ALWAYS change or decontaminate gloves (spray them with Cavicide) when taking hands out of the biosafety cabinet
- Decontaminate any surface, glove gown that may become contaminated by biological material
Always use mechanical pipetting aids.

Don’t:
- Cross hands while working in the biosafety cabinet
- Block front and rear intake grills.
- Reattach gowns, scratch nose/eyes, get hair out of face with gloves on
- Touch equipment with potentially contaminated gloves
- Make sudden swift movement of hands in the biosafety cabinet
- Work in the BSC if alarm or warning light’s on
- DO NOT over fill waste container

8.16.8 Maintenance of the biosafety cabinet

The BSC should be properly cleaned before and after use by surface decontaminating walls, tray and sash with the appropriate disinfectant (cavicide, 70% ethanol). DO NOT use bleach in any concentration in a BSC as it will damage the surfaces. Cavicide is preferred for post-work decontamination.

Once a month the BSC should be “taken apart” and cleaned. To do so after surface decontaminating, lift the tray and remove it from the BSC. Trays are usually heavy and difficult to handle – it is recommended that 2 people perform this step. Clean underneath the tray.

Remove any debris, trash that would have fallen through the grids and vent system (paper towels, glass beads, tubes…). Care should be taken when glass is being used in the BSC, such as Pasteur pipettes, and ampoules, as pieces of glass might be found in the bottom. Always remove glass pieces with tongs, NOT with gloved hands.

Add a generous amount of disinfectant in the bottom of the BSC and let soak. Wipe off the liquid and continue to totally clean the bottom. Clean the walls of the BSC as well as the sash and replace the tray, again 2 people should perform this step. Surface decontaminates the entire BSC one last time. Let the air circulate at least 30 min before starting work in the BSC.

Proper maintenance of the BSC will insure proper functioning as well as limiting contamination of the work.

Remember, always wear 2 pairs of gloves and replace them if they become damaged, and use tongs to handle broken glass.

8.16.9 UV lights in BSC

Certain models of BSC are equipped with UV lights which are used for surface decontamination. If work is properly performed and the BSC is regularly and properly maintained, the use of UV light is not necessary. The use of UV lights in a BSC should be kept to a minimum.

Proper use of the UV lights will ensure safety to the users as well as proper decontamination.
UV light efficiency can be reduced if dust accumulates on the lights; light bulbs need to be regularly cleaned to remove dust. Also, light intensity must be tested, as the efficiency of the UV light will decline before it is visible to the naked eye. Finally, if the UV light is turned on, the area should be unoccupied or the sash completely closed to prevent burns by the UV light.

NEVER work in the BSC if the UV light is turned on, since exposure to UV rays can cause reddening of the skin and eye problems such as photokeratitis and/or conjunctivitis.