Biological Safety Cabinets





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The University of Hong Kong
Safety Office

Biological Safety Cabinets

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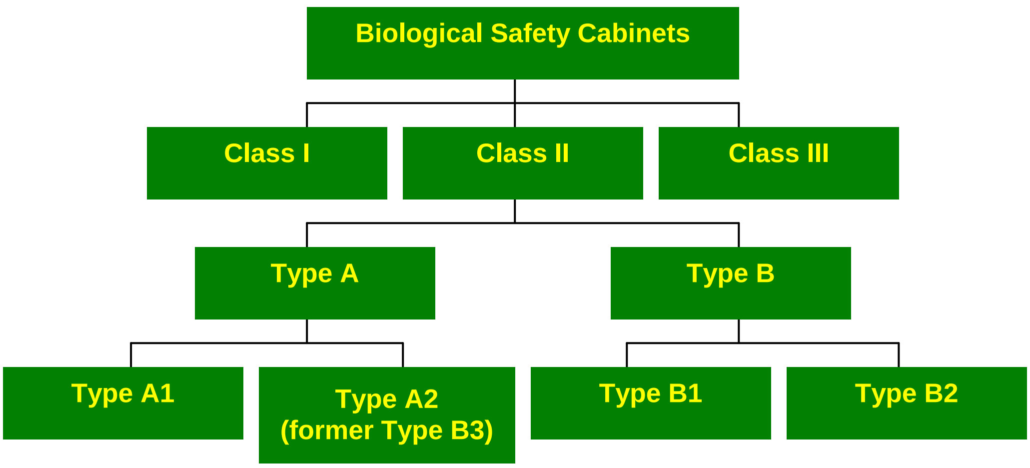
1. Introduction

Staff in biological and biomedical laboratories often need to keep reagents or cultures free from microorganisms. They may also deliberately handle bacteria or viruses for diagnostic or research purposes. Biological safety cabinets are designed for this purpose and will contain any aerosols generated providing protection for both the worker and the environment. The sterile airflow generated within a cabinet also helps to keep reagents and cultures free from unwanted microorganisms. Biological safety cabinets are widely used within the University and there are over 200 spread across various departments mostly in the faculties of Science and Medicine.

To ensure the efficiency and effectiveness of the cabinets' function, there are stringent requirements for installation, testing, decontamination and maintenance. All biological safety cabinets in the University are inspected according to accepted international standards to verify that they are safe in operation. ***The cabinets must be decontaminated before they are moved, before any internal repair, or when filters are to be replaced.***

The air in these cabinets is filtered through a High Efficiency Particulate Air (HEPA) filters and depending on the design type some of the air may be re-circulated. HEPA filters are effective at trapping particulates and infectious agents but not at capturing volatile chemicals or gases. Hence it is not good practice to use Class II biological safety cabinets when working with volatile toxic chemicals and a fume hood or Class I cabinet exhausted to the outside should be used. If it is absolutely necessary to maintain product sterility then small amounts of volatile agents may be used but this must only be in hoods ducted directly to the outside. Class II type B1 and B2 biological safety cabinets do offer protection to the worker but in general these have not been installed in University premises.

There are three classes of Biological Safety Cabinets: I, II, & III as indicated in figure. Class II Biological Safety Cabinet is most common in the University.



Classification of Biological Safety Cabinets

2. Classification of Biological Safety Cabinets

2.1 Class I Biological Safety Cabinet

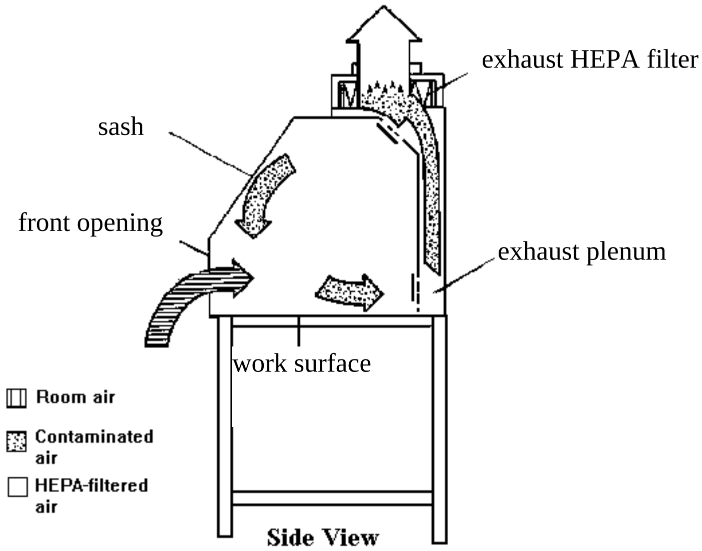


Figure 1: Typical Class I Biological Safety Cabinet

The Class I Biological Safety Cabinet provides protection to the *operator and environment only*. It provides *no protection to the product* (i.e. work inside the cabinet) from contamination.

- The class I Biological Safety Cabinet (Figure 1) is a partially enclosed ventilated cabinet. An air barrier of inward airflow of 0.7–1.0 m/s through the front working opening, across the work space of the cabinet, and out through a HEPA filter, minimizes the escape of airborne aerosols generated within the cabinet.

- Operator protection can be affected by any disturbance to the air barrier such as aerosols releasing from high-energy operations like centrifuges, human activity near the cabinet, closing and opening of a nearby room door or air discharges from a ventilation supply grille located near the cabinet.

- Usually the exhaust air of the Class I biological safety cabinets is discharged outdoors after HEPA filtration.

A Class I Biological Safety Cabinet is suitable for work with low to moderate risk biological agents (i.e. hazard groups 1, 2 & 3). It can be used for work with radionuclides and volatile toxic chemicals.

2.2 Class II Biological Safety Cabinet

The Class II Biological Safety Cabinet provides *protection to the operator, the environment and also the product.*

A class II Biological Safety Cabinet is a ***partially enclosed*** ventilated cabinet. Inward airflow through the front working opening minimizes the escape of aerosols generated within the cabinet to provide operator protection. A Class II cabinet is more susceptible to the effect of operator movement and turbulence within the laboratory than a class I cabinet.

The workspace of the cabinet is flushed with a HEPA filtered laminar downward flow of air for product protection and the exhaust air is also HEPA filtered for environmental protection. The exhaust air may be re-circulated back to the laboratory or ducted to the outdoors. The class II Biological Safety Cabinets are further divided into types A1, A2, B1 and B3.

2.2.1 <u>Characteristics of Class II, type A Biological Safety Cabinet</u>:

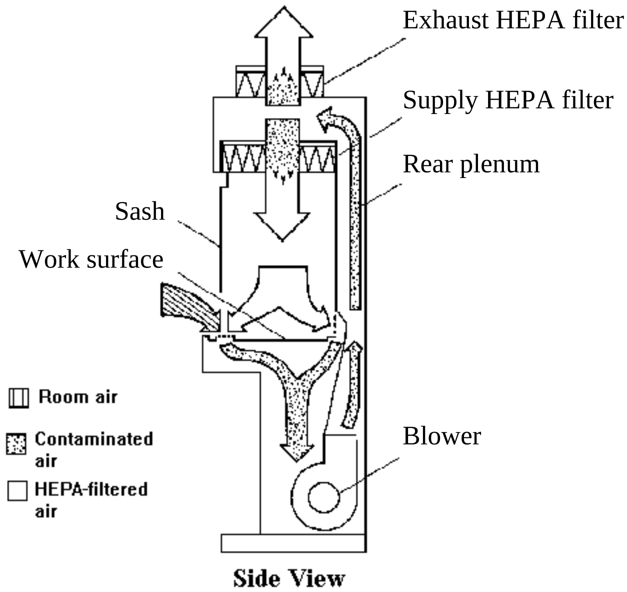


Figure 2: Typical Class II, Type A Biological Safety Cabinet

- Minimum average inflow velocity of 0.38 m/s through the front work access opening.
- Contaminated air, both from the workspace of the cabinet and the room, is drawn in by a fan and is then forced through the downflow supply filter (70%) and the exhaust filter (30%). Hence both the HEPA filtered downflow supply air and exhaust air are from a common plenum (Figure 2).
- Contaminated ducts and plenums may be under positive pressure.
- HEPA filtered air from the cabinet may exhaust back into the laboratory.

The type A Biological Safety Cabinet is suitable for work with low to moderate risk biological agents (hazard group 1, 2 & 3) *in the absence of volatile toxic chemicals and volatile radionuclides*.

2.2.2 Characteristics of Class II, Type A2 Biological Safety Cabinet (Former Type B3):

- An exhausted Type A cabinet maintaining a minimum average inflow velocity of 0.5 m/s through the front work opening.
- HEPA filtered down flow air on the workspace of the cabinet is a portion of the mixed down flow and inflow air from a common plenum.
- All exhaust air is discharged to outdoors by an exhaust fan after HEPA filtration.
- All biological contaminated ducts and plenums are under negative pressure, or surrounded by negative pressure ducts and plenums.

The type A2 Biological Safety Cabinet is suitable for work with low to moderate risk (hazard group 1, 2 and 3) biological agents treated with minute quantities of toxic chemicals and trace quantities of radio-nuclides that will not interfere with the work if re-circulated in the downflow air.

2.2.3 Characteristics of Class II, Type B1 Biological Safety Cabinet:

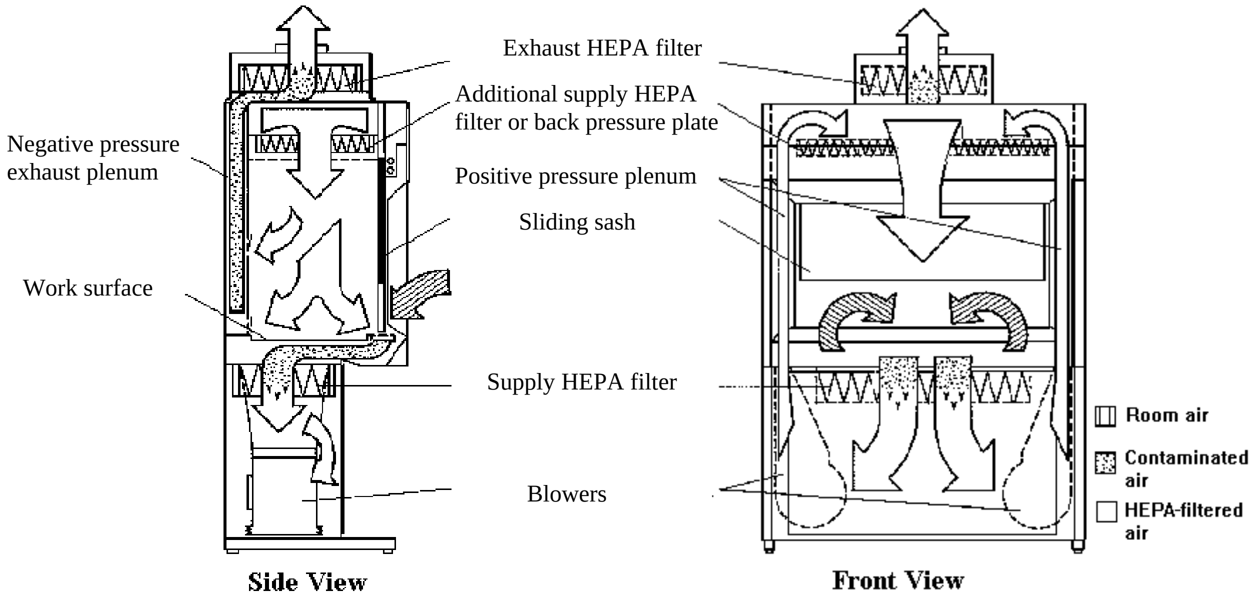


Figure 3: Typical Class II, Type B1 Biological Safety Cabinet

- Intake air velocity at the front work opening is approximately 0.5 m/s.
- About 30% of the contaminated air from the workspace together with inflow room air is filtered through the supply HEPA filter and recirculated to the workspace (Figure 3). About 70% of the air from the work space of the cabinet is drawn through a rear grille and exhausted via a dedicated plenum through an HEPA filter to the outdoors.

- Air re-circulated to the workspace of the cabinet is never mixed with the exhaust air from the cabinet.
- All biological contaminated ducts and plenums are under negative pressure or surrounded by negative pressure ducts and plenums.

The type B1 Biological Safety Cabinet is suitable for work with low to moderate risk (hazard group 1, 2 and 3) biological agents. It may be used with biological agents treated with minute quantities of toxic chemicals and trace amounts of radionuclides if the chemicals or radionuclides will not interfere with the work when re-circulated in the down flow air.

2.2.4 Characteristics of Class II, Type B2 Biological Safety Cabinet (Total Exhaust Cabinet):

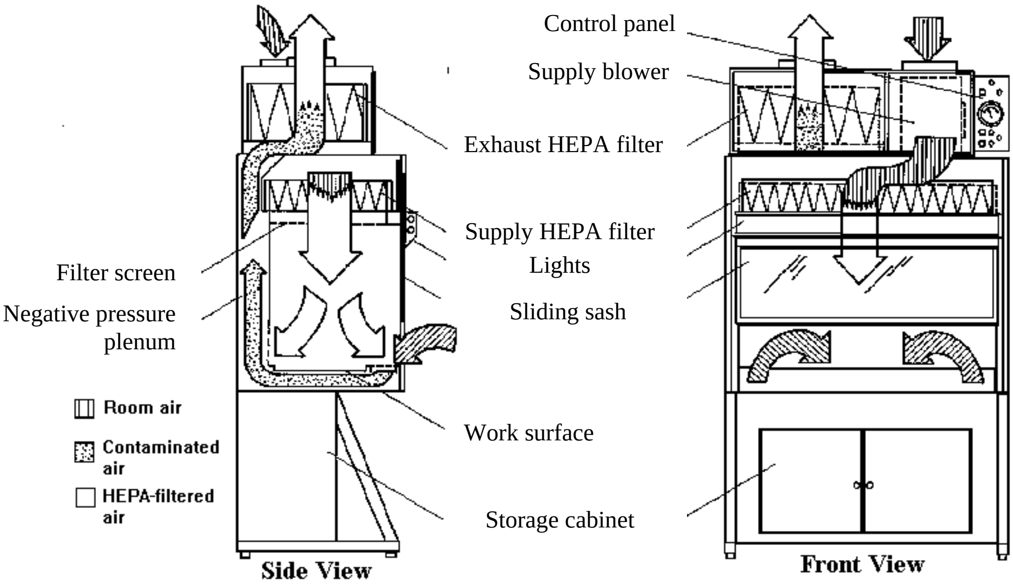


Figure 4: Typical Class II, Type B2 Biological Safety Cabinet

- Minimum average inflow air velocity of 0.5 m/s through the front work access opening.
- No air is re-circulated to the workspace of the cabinet. 100% of air from the workspace of the cabinet is exhausted.
- All HEPA filtered down flow air is drawn in from the laboratory or from outside by a supply fan (Figure 4). All inflow and down flow air from the workspace of the cabinet is exhausted to the outside after filtration through a HEPA filter by an exhaust fan.
- All contaminated ducts and plenums are under negative pressure or surrounded by negative pressure ducts and plenums.
- Interlock system should be installed to prevent the supply fan from operating whenever the exhaust flow is insufficient.

The type B2 Biological Safety Cabinet is suitable for work with low to moderate risk (hazard group 1, 2 and 3) biological agents. It may also be used with biological agents treated with toxic chemicals and radionuclides required in the microbiological studies.

2.3 Class III Biological Safety Cabinet

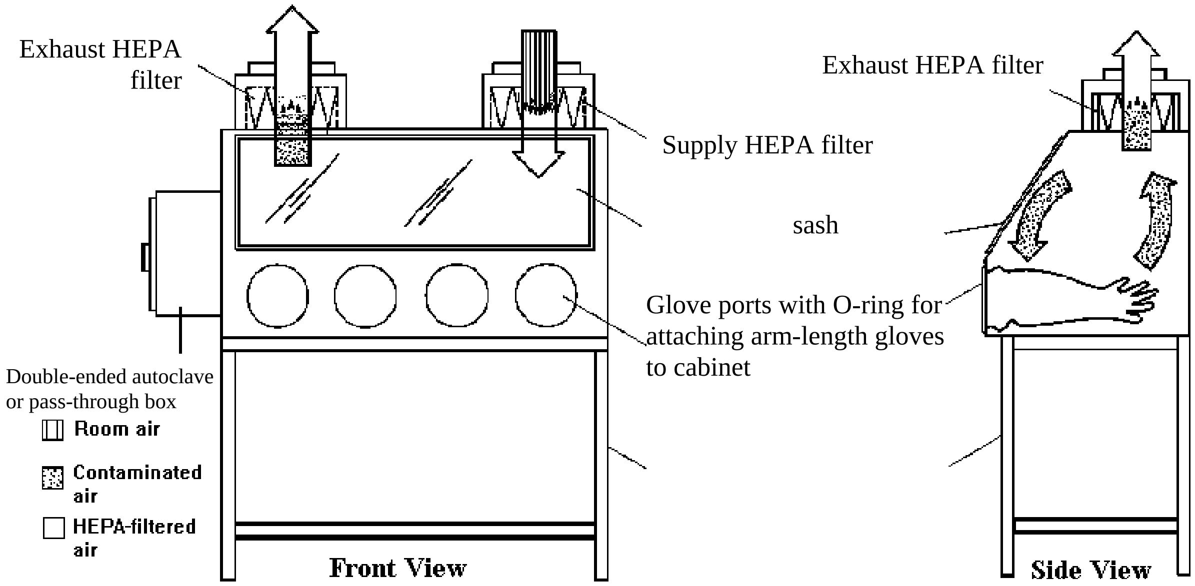


Figure 5: Typical Class III Biological Safety Cabinet

The Class III Biological Safety Cabinet provides the highest level of protection to the operator and the environment.

- The class III Biological Safety Cabinet is a totally enclosed, ventilated cabinet of gas tight construction (Figure 5). The operator is separated from the work by a physical barrier and operations in the cabinet are conducted through attached long-sleeved rubber gloves.
- The cabinet is maintained under negative air pressure, usually about 0.8 inches of water pressure relative to the laboratory.
- Air enters through a HEPA filter and is exhausted through two HEPA filters in series.
- Passage of materials into the cabinet is usually through a sealed airlock, and exit of material may be through an autoclave, a decontamination-type airlock, or a 'dunk-tank' filled with liquid disinfectants.
- Depending on the design of the cabinet, the supply HEPA filter provides particulate-free, but somewhat turbulent airflow within the work environment.
- Flammable gas should not be used because of explosion hazards.

The Class III Biological Safety Cabinet is suitable for work with high-risk biological agents (hazard group 3 and 4). A class III Biological Safety Cabinet should not be used

alone, but should incorporate the necessary equipment and be sited in specially designed laboratory facilities for handling high-risk samples (i.e. containment level 3 or 4). Several Class III cabinets can be joined together in a "line" which is custom-built to provide a larger work area. ***A class III cabinet in basic laboratory should not be used to handle high-risk samples.***

2.4 Cytotoxic Drug Safety Cabinet (CDSC)

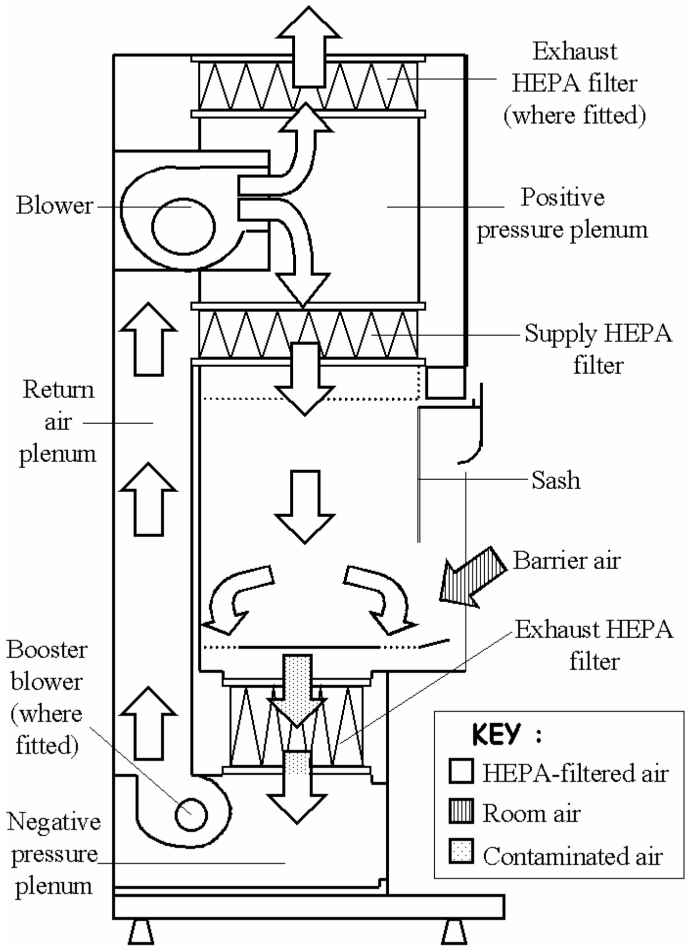


Figure 6 : Typical Cytotoxic Drug Safety Cabinet

The Cytotoxic Drug Safety Cabinet is also a ***partially enclosed*** ventilated cabinet that provides the same operator, product and environment protection as Class II Biological Safety Cabinet. In addition it ***provides protection to the maintenance staff servicing the fans and internal surfaces of the cabinet***.

- The inward airflow at the front work opening offers the operator protection while the HEPA filtered laminar air flow on the work space of the cabinet protects the product from contamination.

- Both the contaminated air from the workspace of the cabinet and the room air from the front work opening mix beneath the work surface and are exhausted through a HEPA filter located directly under the work tray. Hence contaminated air is cleaned first before it reaches the exhaust fan.

- The fans, plenums and internal surfaces are protected from contamination by the cytotoxic drugs handled in the cabinet (Figure 6).

2.5 Horizontal Laminar Flow Clean Bench or Work Station

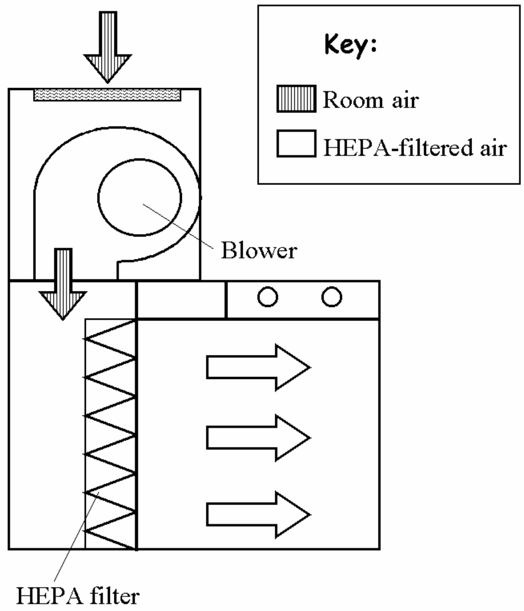


Figure 7 : Horizontal Laminar Flow Clean Work Station

They are not Biological Safety Cabinets, provide only product protection and are not recommended for use in the University because in some instances can increase the risk of infection for the user.

- They discharge HEPA-filtered air across the work surface towards the user.
- They should never be used when handling cell culture materials or drug formulations, or when manipulating potentially infectious materials as the operator is exposed to materials being manipulated in the clean station. Even sterile or other seemingly innocuous biological materials may contain potentially allergenic substances.
- A horizontal clean workstation should never be used as a substitute for a biological safety cabinet (Figure 7).

2.6 Vertical Laminar Flow Clean Bench or Work Station

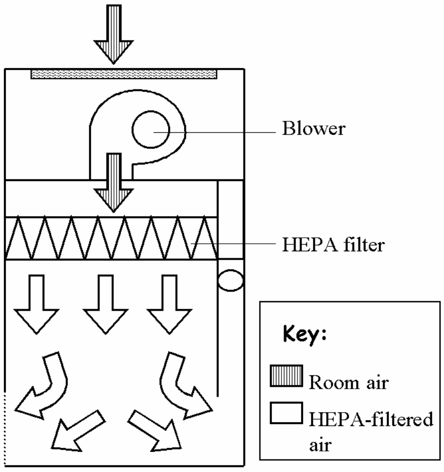


Figure 8 : Vertical Laminar Flow Clean Work Station

They are also not Biological Safety Cabinets, provide only product protection and are not recommended for use in the University because under some circumstances they can increase the risk of infection for the user.

- While they look like a class II safety cabinet with a sash, the air is usually discharged into the room under the sash, resulting in the same potential problems as the horizontal laminar flow clean workstations (Figure 8).

3. Factors to Consider While Choosing a Biological Safety Cabinet (Figure 9)

- The hazard group of the biological agents to be handled in the cabinet.
- The extent to which hazardous aerosols are involved.
- Operations to be conducted in the cabinet. Is protection for the work or product or both required?
- In addition to biological hazards, are radioisotopes, toxic chemicals or carcinogenic materials to be used inside the cabinet?
- The cabinet must meet accepted standards such as British Standard BS5726, NSF-49, European Standard EN12469, Australian Standard AS2252 and AS2567.
- The cabinet must have an airflow indicator with audible alarm for incorrect airflow fitted in a position where it is easily visible to the user of the cabinet.
- Requirement of sufficient make-up air into the laboratory for the exhaust (quite substantial for class I and class IIB2 cabinets) and check whether the current ventilation design is sufficient for this.

Note: Work with category 3 biological hazards required detailed risk assessment for laboratories in which the material is handled may require containment level 3 standard and there are very few facilities at HKU that meet this standard.

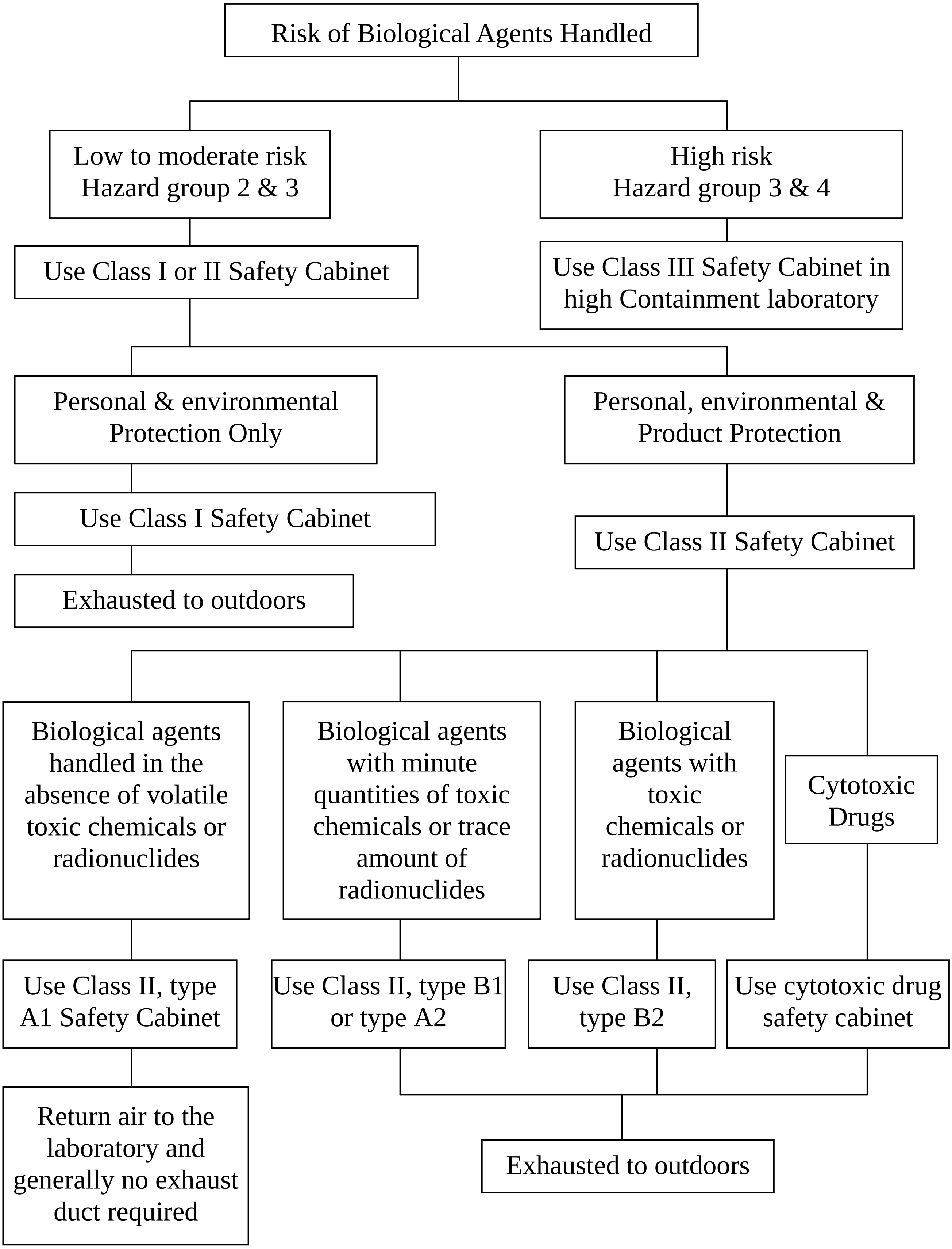


Figure 9: Flowchart of factors involved in choosing a biological safety cabinet.

4. Location of Biological Safety Cabinet in the laboratory

The protection offered by a biological safety cabinet can be severely compromised by its inappropriate location. To prevent this from happening, consideration should be given to the following while selecting a location for a biological safety cabinet.

- The Biological Safety Cabinet unit should be located out of the traffic flow, and away from doors, openable windows, air supply registers, fume hoods or laboratory equipment that creates air movement that could disrupt the containment provided by the inflow air at the front work opening. For further details about the location, please refer to BS 5726: part 2 (1992) or AS2647 (1994).

- Where space permits, a 0.3 m clearance should be provided behind and on each side of the cabinet. Clearance on top of the cabinet should be at least 0.3–0.35 m, preferably 0.6 m, to allow free airflow and for testing and maintenance of the exhaust HEPA filter.

- There should not be any space between the leading edge of the cabinet and the front of the supporting bench.

- The electrical outlet for the cabinet should be accessible for service and electrical safety testing without moving the cabinet.

- There should be sufficient make up air to the room where the cabinets are located if they are exhausted to outdoors.

- For type A biological safety cabinet where exhaust air is re-circulated back to the laboratory, exhaust point such as nearby fume cupboards, open windows should be available for the exhaust of the fumigant after fumigation.

The local installer must verify that satisfactory performance of a biological safety cabinet is obtained on site upon completion of the installation by carrying out the commissioning test.

5. Duct installation for exhaust air

- It is preferable to exhaust the discharge air from the Biological Safety Cabinets to outdoors by a dedicated extraction system. Ducting should be under negative pressure with an exhaust fan sited at the distal end of the ducting with duct length greater than 2 meters or should be air tight with duct length less than 2 meters.

- Where the exhaust of Class II cabinets are exhausted to outdoors, the design of the exhaust ducting system should take into consideration of the testing and maintenance work on the exhaust air and filter. For details of the exhaust methods, please refer to NSF standard 49.

- Adequate space should be provided for the ductwork so that the configuration of the ductwork will not interference with airflow.

- When the cabinet exhaust is ducted to outdoors, the cabinet shall be fitted with an automatic anti-blowback system downstream of the filters to prevent air flowing back into the cabinet as described in BS 5726.

- The discharge should be sited in consideration of the airflow pattern around the building and should be away from the open windows or air intakes of the same and neighbouring buildings. The external wind pressure at the exhaust opening up to 250 Pa should not affect the performance of the cabinet.

6. Certification Tests on the Performance of the Biological Safety Cabinets

6.1 Commissioning test: on-site certification and containment tests must be carried out after installation by the local supplier to verify that the new cabinets are performing to the specification of the manufacturer and are safe for use before it is used.

6.2 Thereafter, each Biological Safety Cabinet should be tested:

- At least annually. More frequent certification may be required for particularly hazardous or critical applications.
- After it has been moved or relocated (even within the same laboratory).
- Increasing numbers of cabinets within a room requires that all cabinets in the room are tested for performance.
- After HEPA filters are changed.
- After maintenance repairs.

6.3 Acceptance should be made with reference to the requirements quoted in the following or other equivalent standards:

- British Standard BS 5726
- National Sanitation Foundation Standard NSF No.49
- Australia Standard AS 2252 and 2567
- European Standard EN 12469

6.4 The following tests are required:

6.4.1 <u>Primary Test</u>

- Down flow Velocity and Volume Test (class II only): to measure the velocity of air moving through the cabinet workspace.
- Inflow (Face) Velocity Test: to determine the calculated or directly measured velocity through the work access opening.
- Airflow Smoke Patterns Tests: to determine if the airflow along the entire perimeter of the work access opening is inward, if airflow within the work area is downward with no dead spots or refluxing, and if there is refluxing to the outside at the window wiper gasket and side seals.
- HEPA Filter Leak Test: to determine the integrity of supply and exhaust HEPA filters, filter housing, and filter mounting frames while the cabinet is operated at the nominal set point velocities.
- Cabinet Leak Tests: to determine if exterior surfaces of all plenums, welds,

gaskets, and plenum penetrations or seals are free of leaks.

- Containment Test (optional): to determine the effectiveness of the cabinet in containing aerosols generated inside the cabinet.
- Alarms and Interlocks Test: to check that the alarms, such as airflow alarms and sash alarms, and interlocks are functional.

6.4.2 Secondary Test

- Lighting Intensity Test: to measure the light intensity on the work surface of the cabinet.
- Noise Level Test: to measure the noise levels produced by the cabinets, as a guide to satisfactory mechanical performance.
- Electrical Leakage and Ground Circuit Resistance and Polarity Tests: to determine if a potential shock hazard exists.
- UV Lamp Test: to test the UV lamp if installed to ensure the germicidal efficacy of the light.

7. General Operating Guidelines for Open-front Biological Safety Cabinets

7.1 Remember:

- Horizontal and vertical laminar flow clean workstations are not microbiological safety cabinets and should not be used as such. The airflow from these stations is outward instead of inward.
- The operator should know the use and limitations of the cabinet in use.
- The cabinet should never be used unless the fan is switched on and the airflow indicator, if present, is in the "safe" position. Any malfunctioning of the cabinet should be reported immediately to the laboratory supervisor.
- If an airflow is activated during the work, immediately cease the work and notify the laboratory supervisor.
- The sash of the cabinet must not be raised when work is in progress in the cabinet.
- Apparatus and materials in the cabinet during operation should be kept to a minimum.
- Centrifuges must not be placed within a Class I or Class II safety cabinet, as they could affect the airflow and efficiency of the cabinet.
- The front grille must not be blocked with papers or equipment items.
- A Bunsen burner should not be used in the cabinet as the heat produced might distort the airflow. The use of disposable plastic loops is recommended.
- Pedestrian traffic in front of the cabinet during work should be minimized as this will not only affect the containment of the cabinet but also disturb the concentration of the operator at work.
- The inspection certificate of the safety cabinet should be checked to make sure that it is regularly inspected with satisfactory results.

7.2 Working Procedures:

- Ensure that the laboratory door is closed.
- Adjust the stool height so that the face of the operator is above the front opening.
- Put on personal protective equipment such as laboratory coats and gloves.
- The Ultraviolet (UV) lamps, if installed inside the cabinet, should be switched off to prevent the ultra-violet light hazard to the operator.
- The cabinet should be switched on for at least 5 minutes before use. Check that the airflow has stabilized in the 'safe' range.
- The work surface should be wiped with appropriate disinfectant as determined by the investigator to meet the requirements of the particular work.
- The surfaces of all materials and containers placed into the cabinet should be wiped with disinfectant to reduce the introduction of contaminants to the cabinet environment.
- All the materials for the proposed work should be placed in the cabinet before the procedure is started to minimize in-and-out motions of the operator's arm, which will affect the containment of the safety cabinet. Movement of arms in-and-out of the cabinet should be slow and perpendicular to the front opening.
- The materials should be carefully arranged so that the contaminated materials will not passed over the clean materials.
- All materials should be placed as far back in the cabinet as practical, toward the rear edge of the work surface and away from the front grille of the cabinet. All operations should be performed at least four inches from the inside edge of the front grille on the work surface.
- Manipulation of materials should be delayed for approximately one minute after placing the hands/arms inside the cabinet to allow the cabinet to stabilize and to "air sweep" the hands and arms to remove surface microbial contaminants.
- Waste materials holder and disinfectant for holding used pipettes should be available inside the cabinet. Bulky items such as biohazard bags, discard pipette trays and suction collection flasks should be placed to one side of the interior of the cabinet.
- Good laboratory practices are still required during the operation in the cabinet. For example, techniques to reduce splatter and aerosol generation will minimize the potential for personnel exposure to infectious materials manipulated within the cabinet.
- Potentially contaminated materials should not be brought out of the cabinet until they have been surface decontaminated or enclosed in a closable container for transfer to an incubator, autoclave or for other decontamination treatment.
- After use, the cabinet surface should be wiped clean with a disinfectant effective against the known and potential biological agents that have been handled.

- The cabinet should be allowed to run for at least 5 minutes, preferably 15 minutes, after the completion of the operation before reuse or switching off.

- The door for the front opening, if available, should be closed before the UV lamps, if installed, is to be switched on.

8. Decontamination of the Biological Safety Cabinets

8.1 Gas decontamination should be carried out:

- before maintenance;
- before certification;
- before the HEPA filters are changed;
- before the cabinet is relocated; or
- when there is a large spill of infectious material in the cabinet.

8.2 A decontamination service for biological safety cabinets is available from the Safety Office.

9. How to handle spillage inside a Biological Safety Cabinet

- Before work commences the operators of the cabinet should formulate the emergency procedures in case of spillage of the agents and make the materials required e.g. disinfectant and auto-clavable plastic bags readily available at any time. Everyone who uses the BSC should have read and understood the procedures.

- In case of spillage inside the cabinet, the cabinet should continue to operate and an appropriate disinfectant against the agents should be used for the clean up as soon as possible.

- The operator should put on protective gloves and place paper towels soaked with the appropriate disinfectant to cover up the spillage. Generation of aerosols during the process should be minimized.

- Sufficient contact time with the disinfectant should be allowed before the paper towels are collected into auto-clavable bag.

- For spills of moderate- or high-risk agents, all reachable cabinet surfaces should be wiped with the disinfectant.

- All items within the cabinet should be packed for sterilization by autoclave, and for items that cannot be autoclaved; the surfaces should be wiped carefully with disinfectant.

- The operator should change his/her protective clothing and gloves and treat them as contaminated materials after the clean up process.

- Hands should be washed whenever gloves are changed or removed.

- The cabinet should be allowed to run for at least 15 minutes before operation is resumed in the cabinet.

- Decontamination of the cabinet by formaldehyde is required for a large spillage of a moderate-risk agent (hazard group 2 and 3).

10. References

- British Standard BS5726 (1992): Microbiological Safety Cabinets.
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- European Committee for Standardization (2000) European Standard EN12469: Biotechnology – Performance criteria for microbiological safety cabinets.
- Categorisation of biological agents according to hazard and categories of containment (1995) Advisory Committee on Dangerous Pathogen, HSE.