Updates on the Use of Biosafety Cabinet in Different Bio Containment Levels

The Basics

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PAKISTAN BIOLOGICAL SAFETY ASSOCIATION

Four Primary Controls of Biosafety

Engineering	Personal Protective Equipment (PPE)	Standard Operating Procedures (SOPs)	Leadership
Locks on doors	Gloves	Emergency Evacuation	Training
Directional Airflow	Eye Protection	Waste Disposal	Vaccinations
Interlocked Doors	Laboratory Coat	Spill Cleanup	SOP Compliance
Biosafety Cabinets	N95	Needle stick	Surveillance (M/I)
Autoclaves	Booties	Lab Decon	SOP Evaluation/
HEPA Filters	PAPR	Medical Emergencies	Background Checks

One control is not more important than the other

Risk Group Categories

RISK GROUP CATEGORIES

- **The principal hazardous characteristics of an agent** are: its **capability to infect** and **cause disease in a susceptible human** or animal host, its **virulence** as measured by the severity of disease, and the **availability of preventive measures** and **effective treatments for the disease**

- The World Health Organization (WHO) has recommended an agent risk group classification for laboratory use that describes **four general risk groups** based on these principal characteristics and the route of transmission of the natural disease.

- The four groups address the risk to both the laboratory worker and the community.

RISK GROUP 1

(No or low individual and community risk)

A microorganism unlikely to cause human or animal disease.

RISK GROUP 2

(Moderate individual risk; low community risk)

A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but

Effective treatment and preventive measures are available and the risk of spread of infection is limited.

RISK GROUP 3

(High individual risk; low community risk)

A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another.

Effective treatment and preventive measures are available.

RISK GROUP 4

(High individual and community risk)

A pathogen that usually causes serious human or animal disease and can be readily transmitted from one individual to another, directly or indirectly.

Effective treatment and preventive measures are not usually available

Just Think !!!

AGENT	RISK GROUP	PROCESS	LOCATION	BIOSAFETY LEVEL
Ebola Virus		Working with attenuated strains	Pakistan	
HIV		High quantities and low virulence	West Africa	
HIV		High quantities and low virulence	United States	
Hepatitis B Virus		Research	United States	
Hepatitis B Virus		Research	Pakistan	
Polio Virus		Clinical	Afghanistan	
Polio Virus		Clinical	UK	

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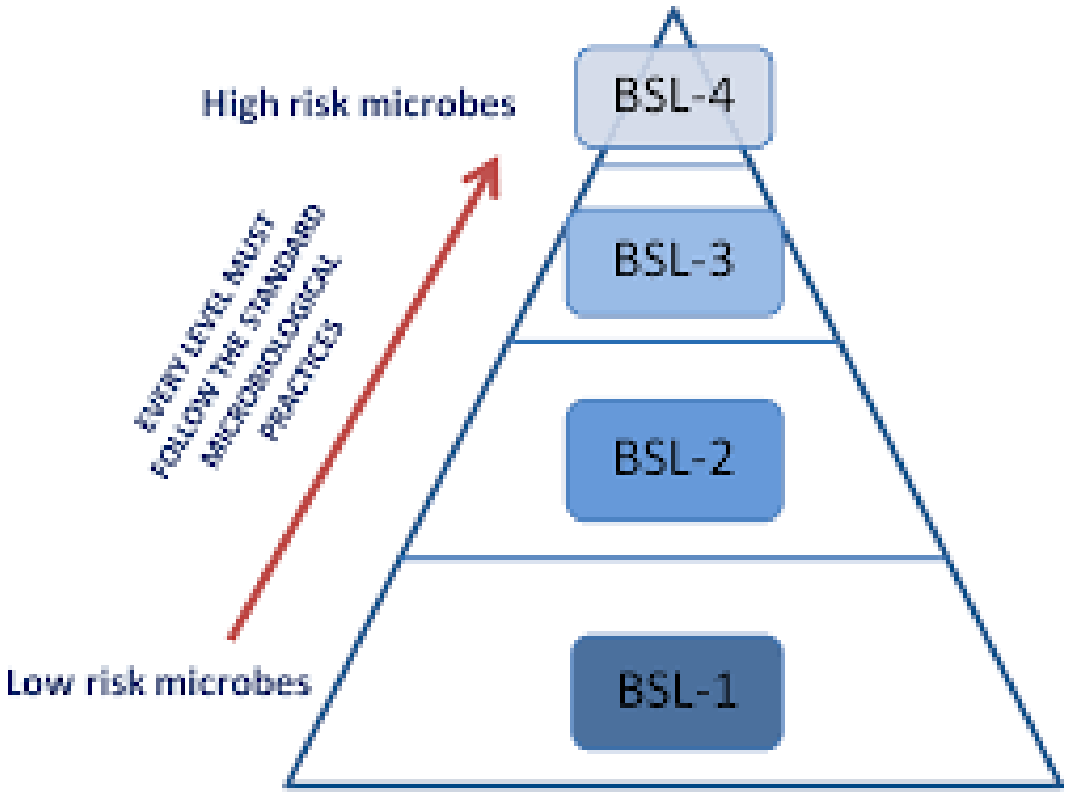
RISK GROUP CATEGORY CONCLUSIONS

- It is a possibility that **Risk Group Categories may no longer exist** within the new biosafety guideline publications (WHO and BMBL).

- Biosafety processes should always be **based on risk assessments** – which included the identification of hazards which are different based on location of country, resources available, staff expertise, organizational culture, access to vaccines and prophylactic countermeasures, status of agent (live or attenuated), and what is being done with the agent within the laboratory.

The sole reliance on risk group categories to assign biosafety levels leads to gross assumptions which may lead to excessive waste of valuable resources.

Containment Levels



BIOSAFETY LEVEL 1 (BSL1)

1. Laboratories **should** have doors for access control.

2. Laboratories **must** have a sink for hand washing.

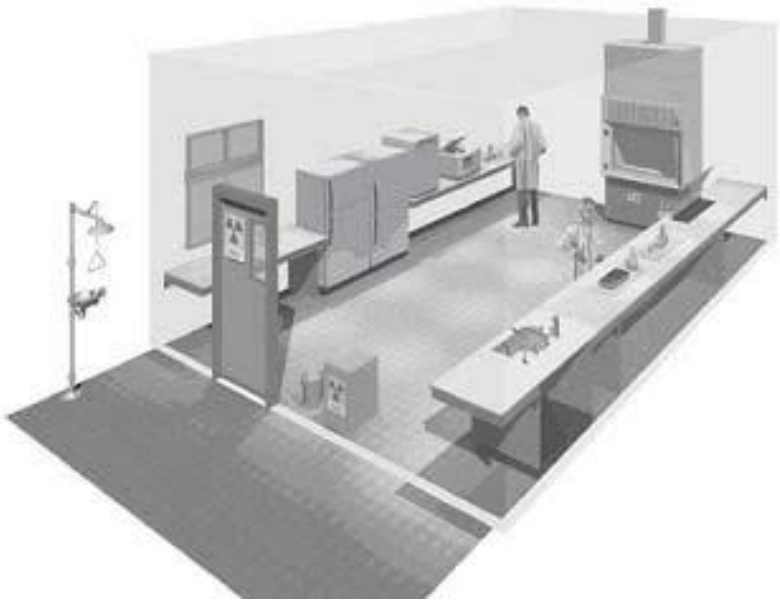
3. The laboratory **should** be designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.

4. Laboratory furniture **must** be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment **should** be accessible for cleaning.

   a) Bench tops **must** be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

   b) Chairs used in laboratory work **must** be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

5. Laboratories windows that open to the exterior **should** be fitted with screens.



BIOSAFETY LEVEL 2 (BSL 2)

1. Laboratory doors **should** be self-closing and have locks in accordance with the institutional policies.

2. Laboratories **must** have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It **should** be located near the exit door.

3. The laboratory **should** be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.

4. Laboratory furniture **must** be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment **should** be accessible for cleaning.

   a) Bench tops **must** be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

   b) Chairs used in laboratory work **must** be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

5. Laboratory windows that open to the exterior are not **recommended**. However, if a laboratory does have windows that open to the exterior, they **must** be fitted with screens.

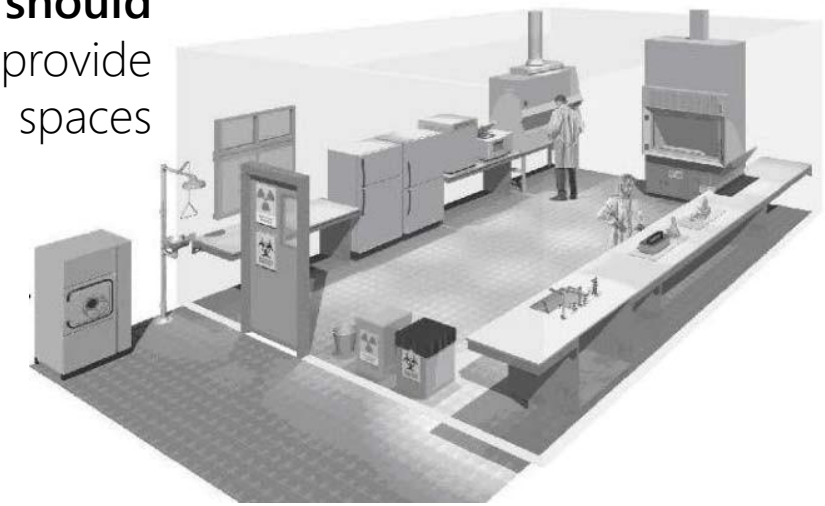
BIOSAFETY LEVEL 2 (BSL 2)

6. Properly maintained BSCs, other PPE, or other physical containment devices must be used whenever high concentrations or volumes are being used in the laboratory or procedures with potential of causing aerosols or splashes. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.

7. Vacuum lines **should** be protected with liquid disinfectant traps.

8. An eyewash station **must** be readily available.

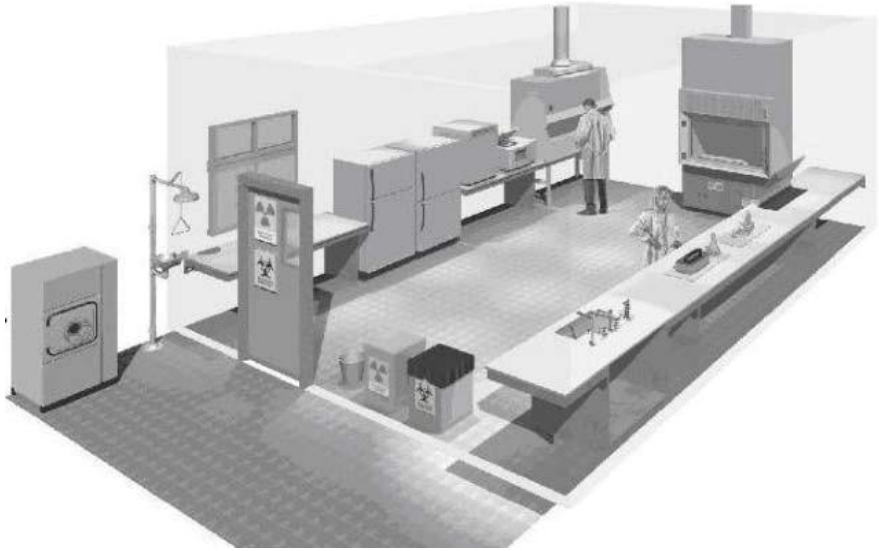
9. There are no specific requirements for ventilation systems. However, planning of new facilities **should** consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.



BIOSAFETY LEVEL 2 (BSL 2)

10. HEPA filtered exhaust air from a Class II BSC can be safely recirculation back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly exhausted to the outside through a hard connection. Provisions to assure proper safety cabinet performance and air system operation **must** be verified.

11. A method for decontaminating all laboratory wastes **should** be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).



BIOSAFETY LEVEL 3 (BSL 3)

1. Laboratory doors **must** be self-closing and have locks in accordance with the institutional policies. The laboratory **must** be separated from areas that are open to unrestricted traffic flow within the building. Laboratory access **is** restricted. Access to the laboratory **is** through two self-closing doors. A clothing change room (anteroom) may be included in the passageway between the two self-closing doors.

2. Laboratories **must** have a sink for hand washing. The sink **must** be hands-free or automatically operated. It **should** be located near the exit door. If the laboratory is segregated into different laboratories, a sink **must** also be available for hand washing in each zone. Additional sinks may be **required** as determined by the risk assessment.

3. The laboratory **must** be designed so that it can be easily cleaned and decontaminated. Carpets and rugs are not permitted. Seams, floors, walls, and ceiling surfaces **should** be sealed. Spaces around doors and ventilation openings **should** be capable of being sealed to facilitate space decontamination.

   a) Floors **must** be slip resistant, impervious to liquids, and resistant to chemicals. Consideration **should** be given to the installation of seamless, sealed, resilient or poured floors, with integral cove bases.

   b) Walls **should** be constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.

   c) Ceilings **should** be constructed, sealed, and finished in the same general manner as walls.

BIOSAFETY LEVEL 3 (BSL 3)

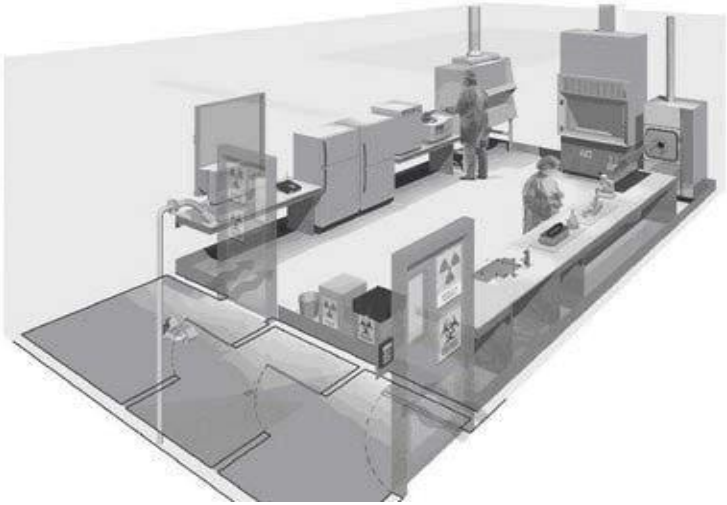
Decontamination of the entire laboratory **should** be considered when there has been gross contamination of the space, significant changes in laboratory usage, for major renovations, or maintenance shut downs.
Selection of the appropriate materials and methods used to decontaminate the laboratory **must** be based on the risk assessment.

4. Laboratory furniture **must** be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment **must** be accessible for cleaning.

a) Bench tops **must** be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

b) Chairs used in laboratory work **must** be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

5. All windows in the laboratory **must** be sealed.

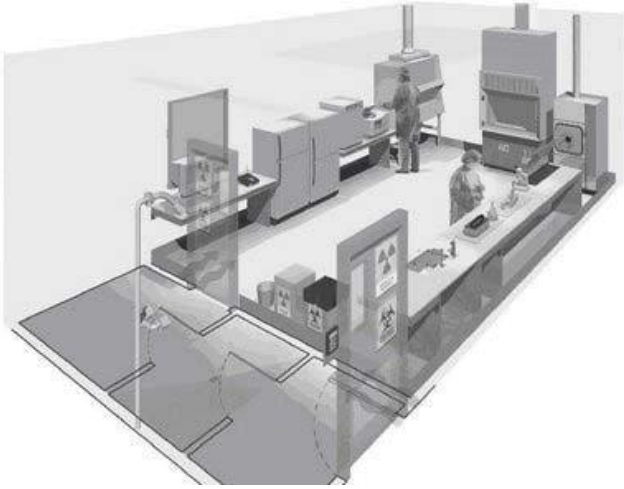


BIOSAFETY LEVEL 3 (BSL 3)

6. BSCs **must** be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs **should** be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

7. Vacuum lines **must** be protected with HEPA filters, or their equivalent. Filters **must** be replaced as needed. Liquid disinfectant traps may be **required**.

8. An eyewash station **must** be readily available in the laboratory.



9. A ducted air ventilation system is **required**. This system **must** provide sustained directional airflow by drawing air into the laboratory from "clean" areas toward "potentially contaminated" areas. The laboratory **shall** be designed such that under failure conditions the airflow will not be reversed.

   a) Laboratory personnel **must** be able to verify directional airflow. A visual monitoring device, which confirms directional airflow, **must** be provided at the laboratory entry. Audible alarms **should** be considered to notify personnel of air flow disruption.

   b) The laboratory exhaust air **must** not re-circulate to any other area of the building.

   c) The laboratory building exhaust air **should** be dispersed away from occupied areas and from building air intake locations or the exhaust air **must** be HEPA filtered.

BIOSAFETY LEVEL 3 (BSL 3)

10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly exhausted to the outside through a hard connection. Provisions to assure proper safety cabinet performance and air system operation **must** be verified. BSCs **should** be certified at least annually to assure correct performance. Class III BSCs **must** be directly (hard) connected up through the second exhaust HEPA filter of the cabinet. Supply air **must** be provided in such a manner that prevents positive pressurization of the cabinet.

11. A method for decontaminating all laboratory wastes **should** be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, or other validated decontamination method).

12. Equipment that may produce infectious aerosols **must** be contained in primary barrier devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters **should** be tested and/or replaced at least annually.

13. Facility design consideration **should** be given to means of decontaminating large pieces of equipment before removal from the laboratory.

14. Enhanced environmental and personal protection may be **required** by the agent summary statement, risk assessment, or applicable local, state, or federal regulations. These laboratory enhancements may include, for example, one or more of the following: an anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities; gas tight dampers to facilitate laboratory isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; and advanced access control devices, such as biometrics.

15. The BSL-3 facility design, operational parameters, and procedures **must** be verified and documented prior to operation. Facilities **must** be re-verified and documented at least annually.

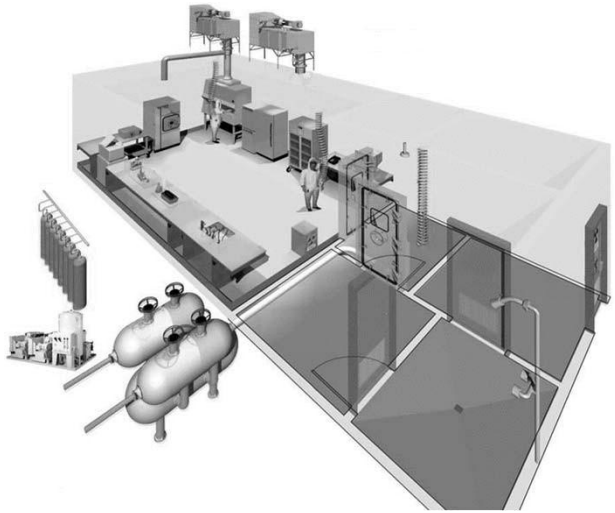
BIOSAFETY LEVEL 4 (BSL 4)

1. The BSL-4 suit laboratory consists of either a separate building or a clearly demarcated and isolated zone within a building. Laboratory doors **must** have locks in accordance with the institutional policies.

   Rooms in the facility **must** be arranged to ensure exit by sequential passage through the chemical shower, inner (dirty) change room, personal shower, and outer (clean) changing area.

   Entry into the BSL-4 laboratory **must** be through an airlock fitted with airtight doors. Personnel who enter this area **must** wear a positive pressure suit supplied with HEPA filtered breathing air. The breathing air systems **must** have redundant compressors, failure alarms and emergency backup.

   A chemical shower **must** be provided to decontaminate the surface of the positive pressure suit before the worker leaves the laboratory. In the event of an emergency exit or failure of the chemical shower system, a method for decontaminating positive pressure suits, such as a gravity fed supply of chemical disinfectant, **is** needed.



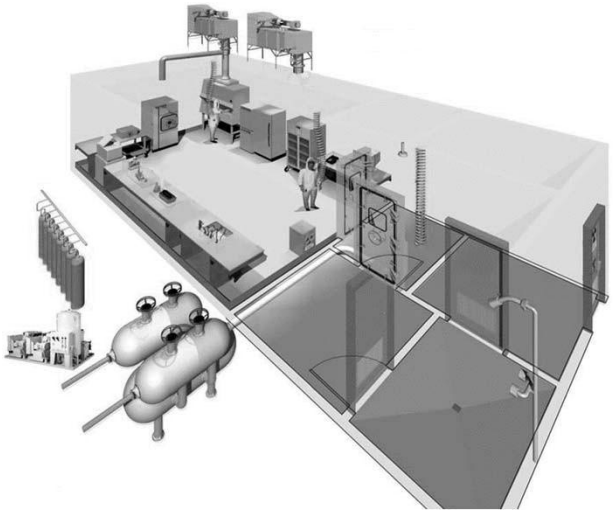
BIOSAFETY LEVEL 4 (BSL 4)

An automatically activated emergency power source **must** be provided, at a minimum, for the laboratory exhaust system, life support systems, alarms, lighting, entry and exit controls, BSCs, and door gaskets.

Monitoring and control systems for air supply, exhaust, life support, alarms, entry and exit controls, and security systems **should** be on a UPS.

A double-door autoclave, dunk tank, or fumigation chamber **must** be provided at the containment barrier for the passage of materials, supplies, or equipment in or out of the laboratory.

2. Sinks inside the suit laboratory **should** be placed near procedure areas and be connected to the wastewater decontamination system.



BIOSAFETY LEVEL 4 (BSL 4)

3. Walls, floors, and ceilings of the laboratory **must** be constructed to form a sealed internal shell to facilitate fumigation and prohibit animal and insect intrusion. The internal surfaces of this shell **must** be resistant to chemicals used for cleaning and decontamination of the area. Floors **must** be monolithic, sealed and coved.

   All penetrations in the internal shell of the laboratory, suit storage room and the inner change room **must** be sealed.

   Drains, if present, in the laboratory floor **must** be connected directly to the liquid waste decontamination system. Sewer vents **must** have protection against insect and animal intrusion.

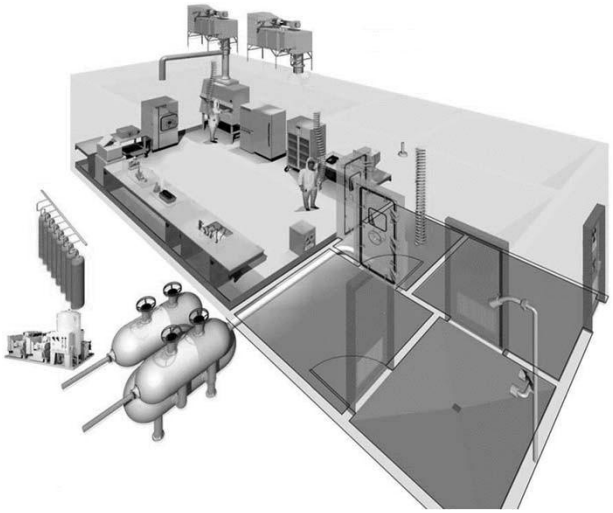
   Services and plumbing that penetrate the laboratory walls, floors, or ceiling **must** be installed to ensure that no backflow from the laboratory occurs. These penetrations **must** be fitted with two (in series) backflow prevention devices. Consideration **should** be given to locating these devices outside of containment. Atmospheric venting systems **must** be provided with two HEPA filters in series and be sealed up to the second filter.

4. Laboratory furniture **must** be of simple construction, capable of supporting anticipated loading and uses. Sharp edges and corners **should** be avoided. Spaces between benches, cabinets, and equipment **must** be accessible for cleaning and decontamination. Chairs and other furniture **must** be covered with a non-porous material that can be easily decontaminated.

5. Windows **must** be break-resistant and sealed.

6. BSCs and other primary containment barrier systems **must** be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs **should** be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

7. Central vacuum systems are not **recommended**. If, however, there is a central vacuum system, it **must** not serve areas outside the BSL-4 laboratory. Two in-line HEPA filters **must** be placed near each use point. Filters **must** be installed to permit in-place decontamination and replacement.



BIOSAFETY LEVEL 4 (BSL 4)

8. An eyewash station **must** be readily available in the laboratory area for use during maintenance and repair activities.

9. A dedicated, non-recirculating ventilation system **is** provided. Only laboratories with the same HVAC requirements (i.e., other BSL-4 labs, ABSL-4, BSL-3 Ag labs) may share ventilation systems if gas-tight dampers and HEPA filters isolate each individual laboratory system.

   The supply and exhaust components of the ventilation system **must** be designed to maintain the laboratory at negative pressure to surrounding areas and provide differential pressure or directional airflow as appropriate between adjacent areas within the laboratory.

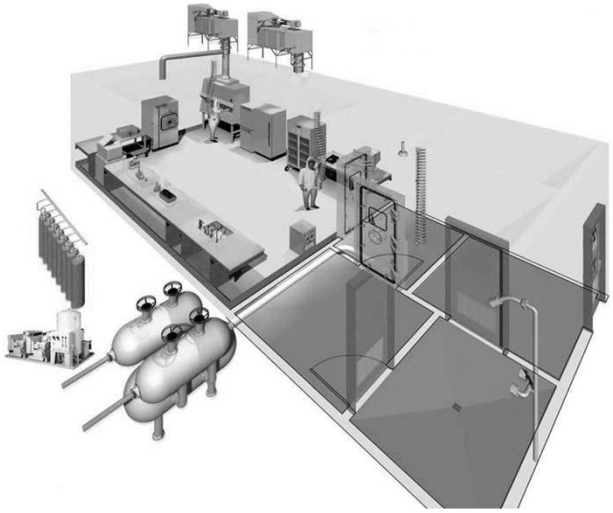
   Redundant supply fans are **recommended**. Redundant exhaust fans are **required**. Supply and exhaust fans **must** be interlocked to prevent positive pressurization of the laboratory.

   The ventilation system **must** be monitored and alarmed to indicate malfunction or deviation from design parameters. A visual monitoring device **must** be installed near the clean change room so proper differential pressures within the laboratory may be verified prior to entry.

   Supply air to the laboratory, including the decontamination shower, **must** pass through a HEPA filter. All exhaust air from the suit laboratory, decontamination shower and fumigation or decontamination chambers **must** pass through two HEPA filters, in series, before discharge to the outside. The exhaust air discharge **must** be located away from occupied spaces and air intakes.

   All HEPA filters **must** be located as near as practicable to the laboratory in order to minimize the length of potentially contaminated ductwork. All HEPA filters **must** be tested and certified annually.

   The HEPA filter housings **must** be designed to allow for in situ decontamination and validation of the filter prior to removal. The design of the HEPA filter housing **must** have gas-tight isolation dampers, decontamination ports, and ability to scan each filter assembly for leaks.



BIOSAFETY LEVEL 4 (BSL 4)

10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet **is** tested and certified at least annually and operated according to the manufacturer's recommendations. Biological safety cabinets can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation **must** be verified.

11. Pass through dunk tanks, fumigation chambers, or equivalent decontamination methods **must** be provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the BSL-4 laboratory. Access to the exit side of the pass-through **shall** be limited to those individuals authorized to be in the BSL-4 laboratory.

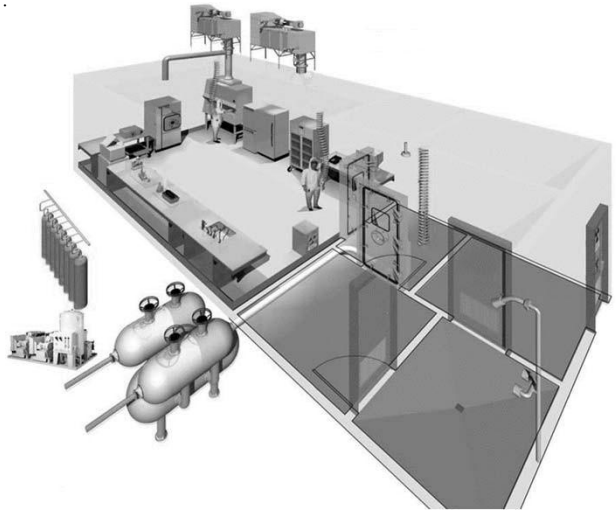
12. Liquid effluents from chemical showers, sinks, floor drains, autoclave chambers, and other sources within the laboratory **must** be decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer.

    Decontamination of all liquid wastes **must** be documented. The decontamination process for liquid wastes **must** be validated physically and biologically. Biological validation **must** be performed annually or more often if **required** by institutional policy.

    Effluents from personal body showers and toilets **may** be discharged to the sanitary sewer without treatment.

13. A double-door, pass through autoclave(s) **must** be provided for decontaminating materials passing out of the cabinet laboratory. Autoclaves that open outside of the laboratory **must** be sealed to the interior wall. This bioseal **must** be durable, airtight, and capable of expansion and contraction. Positioning the bioseal so that the equipment can be accessed and maintained from outside the laboratory is strongly **recommended**. The autoclave doors **must** be interlocked so that only one can be opened at any time and be automatically controlled so that the outside door to the autoclave can only be opened after the decontamination cycle has been completed.

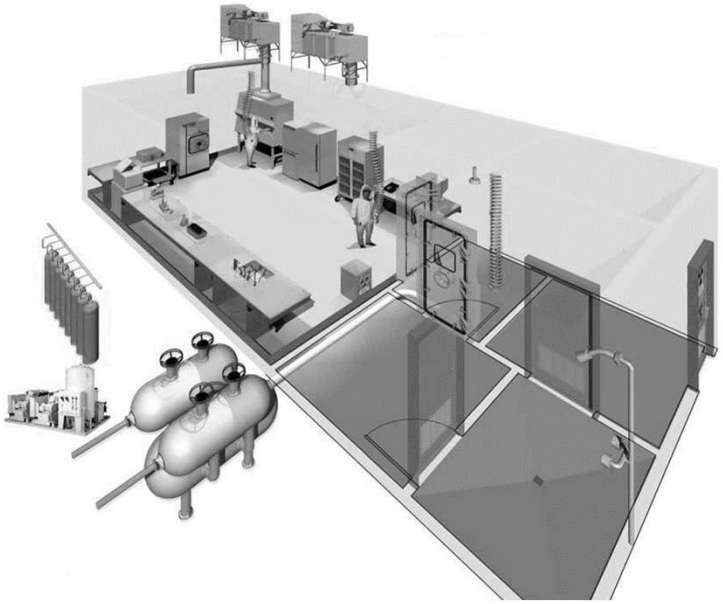
    Gas and liquid discharge from the autoclave chamber **must** be decontaminated. When feasible, autoclave decontamination processes **should** be designed so that unfiltered air or steam exposed to infectious material cannot be released to the environment.



BIOSAFETY LEVEL 4 (BSL 4)

14. The BSL-4 facility design parameters and operational procedures **must** be documented. The facility **must** be tested to verify that the design and operational parameters have been met prior to operation. Facilities **must** also be re-verified annually. Verification criteria **should** be modified as necessary by operational experience.

15. Appropriate communication systems **must** be provided between the laboratory and the outside (e.g., voice, fax, and computer). Provisions for emergency communication and emergency access or egress **must** be developed and implemented.



Testing your Knowledge

Scenario 1	Scenario 2	Scenario 3
Autoclave within the facility	Sequential passage through inner change room	No sink for hand washing
Self closing doors	Personal showers and outer change room	Labs furniture capable of supporting anticipated loads and uses
Process for sealing labs	Windows are break resistant	Bench top impervious to water and resistant to heat
Slip resistant floor	Double door autoclave inside lab	Enough light to perform various activities
Screens on windows that open to exterior	Single HEPA filter exhaust	Chair covered with a non-porous material capable of cleaning and decontamination
Biosafety Cabinet properly installed	Single HEPA filter supply	-
Eye Wash Station in lab	Liquid decontamination process before release to sanitary	-
Directional air flow	Be completely sealed	-
WHAT IS THE BIOSAFETY LEVEL FOR THE ENGINEERING CONTROLS DESCRIBED ABOVE?		

BIOSAFETY CABINET TYPES

Primary

(Safety Equipment)

- Personal Protective Equipment (PPE)
- Biosafety Cabinets (BSC)
- Pipetting Devices
- Centrifuges
- Freezers and Incubators
- Autoclaves

Secondary

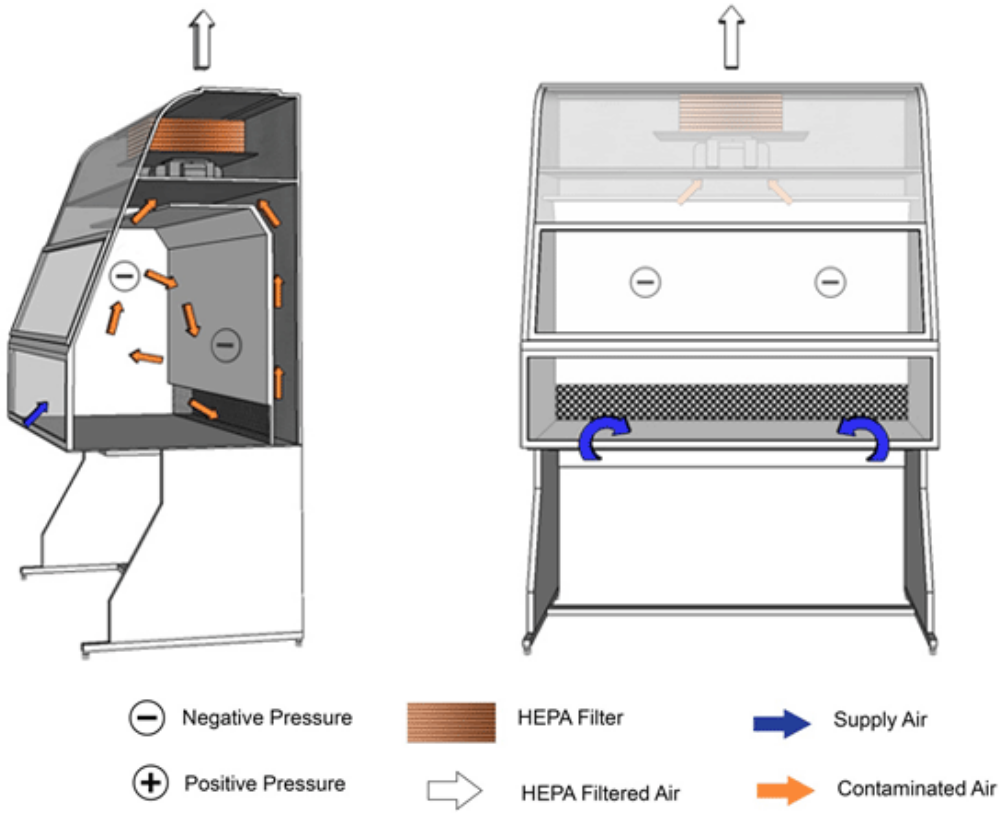
(Facility Design)

- Directional Airflow
- Interlocking/Self Closing Doors
- Laboratory design (placement of sinks, eyewash stations, biosafety cabinets, autoclaves)
- Security of laboratory and agents
- Double-door Entry

PAKISTAN BIOLOGICAL SAFETY ASSOCIATION

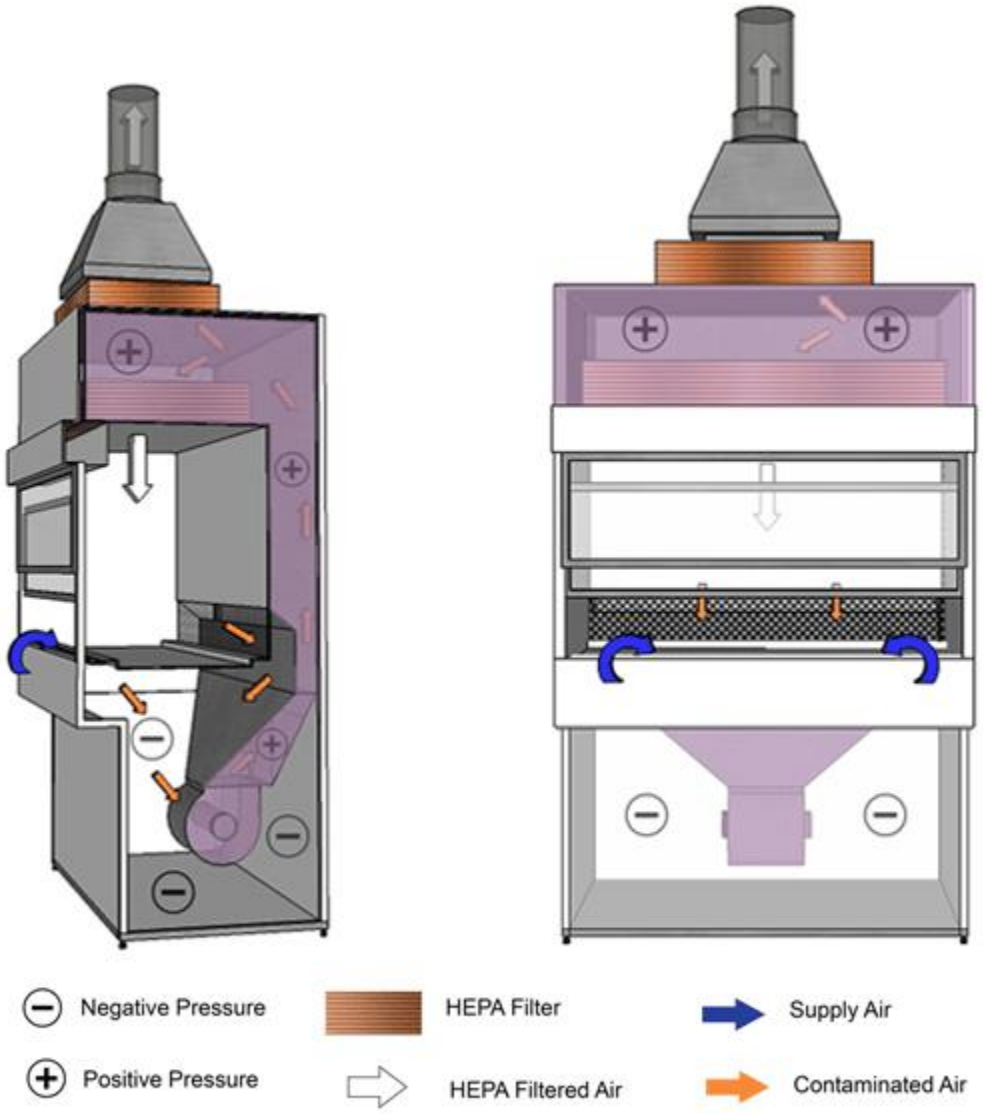
CLASS I BSC

- Protects person and environment.
- **DOES NOT** protect product.
- Protects the person with 75 lfpm directional airflow into the cabinet.
- Protects the environment with HEPA filtered exhaust.
- 70% recirculation – 30% exhaust.



CLASS II BSC A1

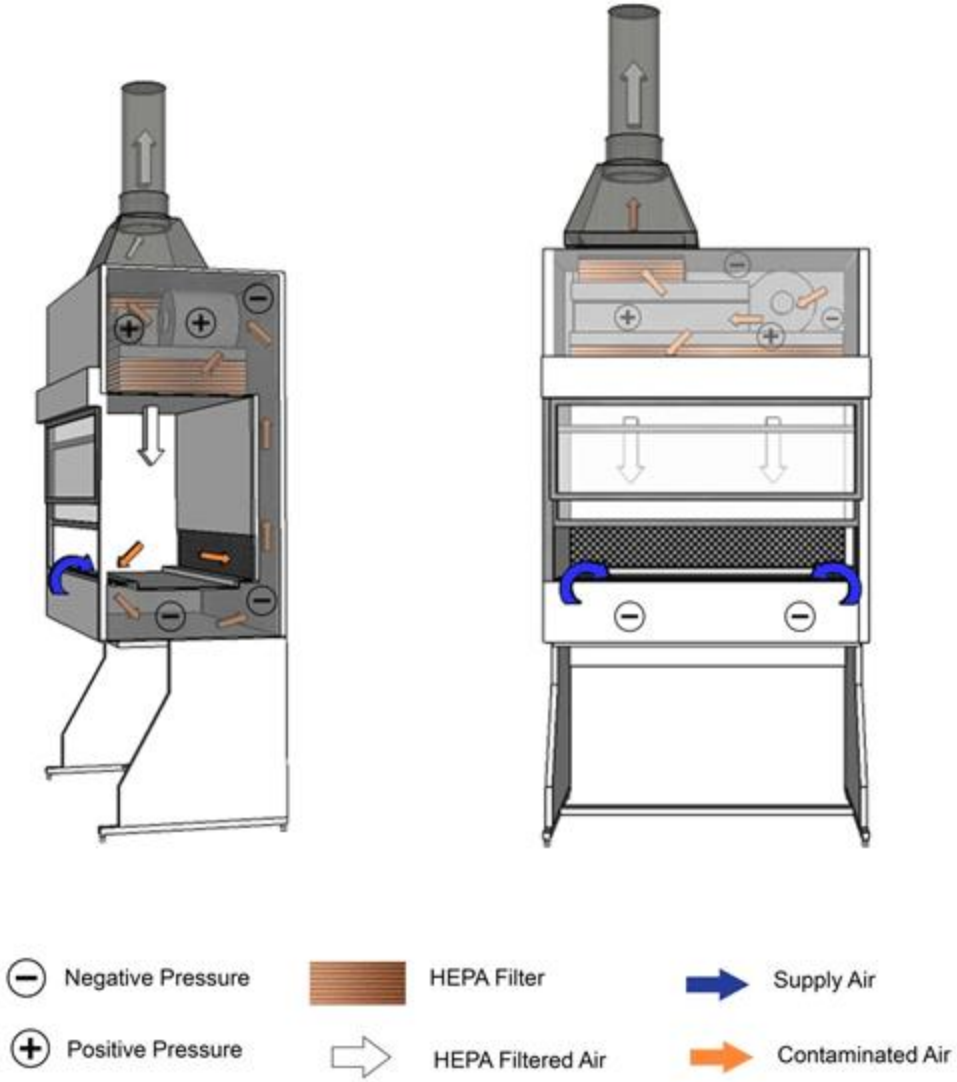
- Protects person, product and environment.
- + pressure plenum.
- Protects the person with 75 lfpm directional airflow into the cabinet.
- Protects the environment with HEPA filtered exhaust.
- Protects the product with HEPA filtered laminar airflow.
- 70% recirculation – 30% exhaust.



Cabinet exhaust may be recirculated into the room or vented to the outside atmosphere through an air gap type (thimble) connection.

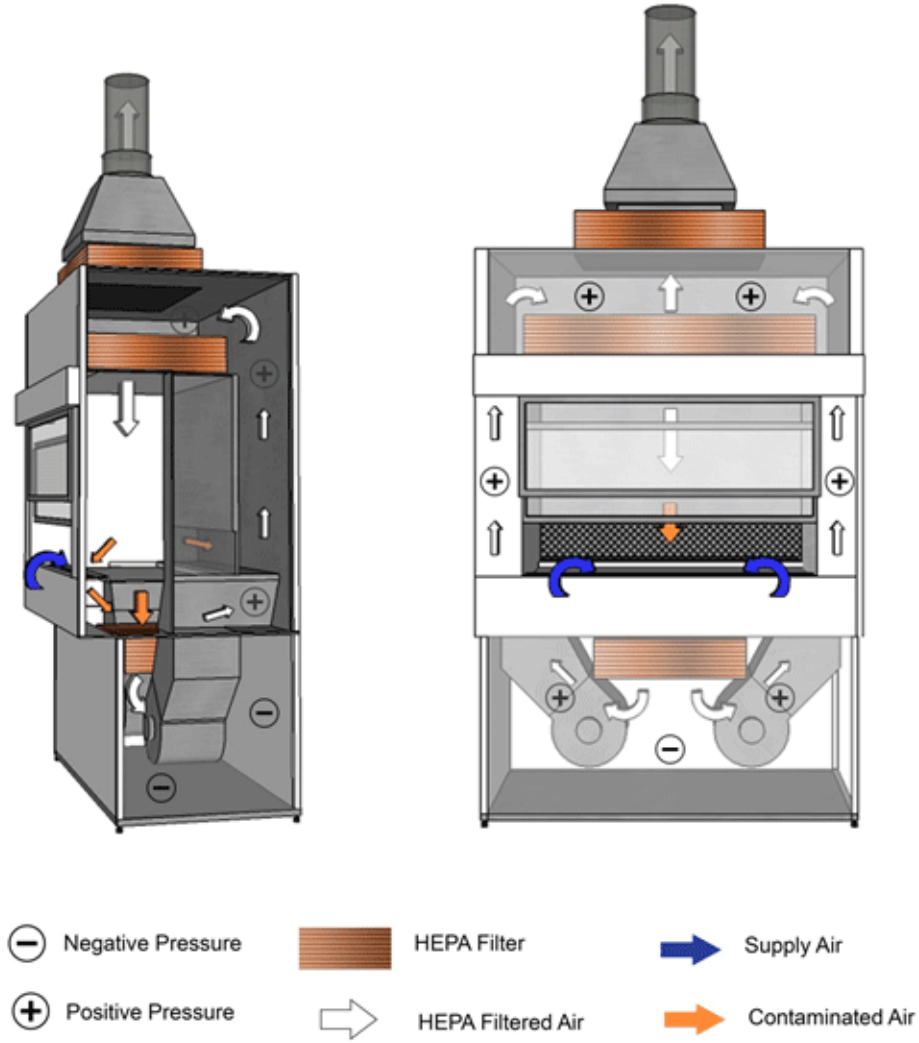
CLASS II BSC A2

- Protects person, product and environment.
- - pressure plenum.
- Protects the person with 100 lfpm directional airflow into the cabinet.
- Protects the environment with HEPA filtered exhaust.
- Protects the product with HEPA filtered laminar airflow.
- 70% recirculation – 30% exhaust.



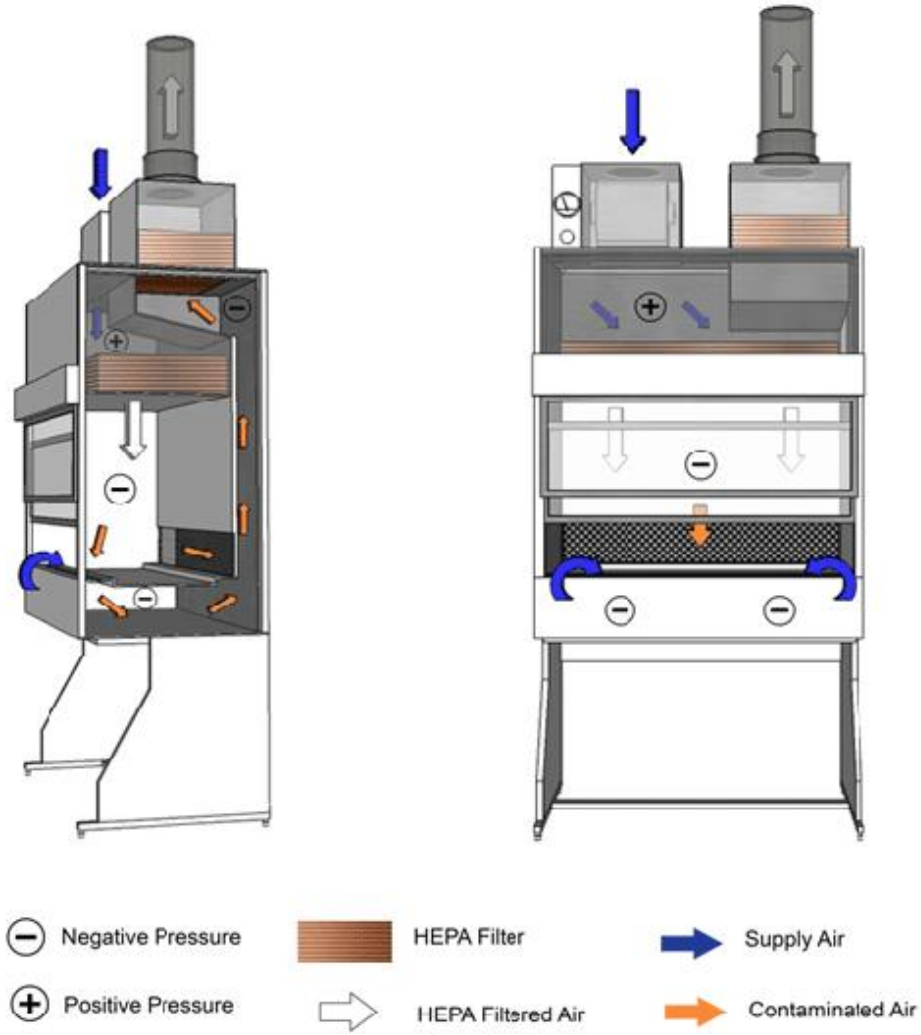
CLASS II BSC B1

- Protects person, product and environment
- **Must be hard-ducted** **(can work with minute amounts of chemicals)**
- Protects the person with 100 lfpm directional airflow into the cabinet.
- Protects the environment with HEPA filtered exhaust.
- Protects the product with HEPA filtered laminar airflow.
- 30% recirculation – 70% exhaust.

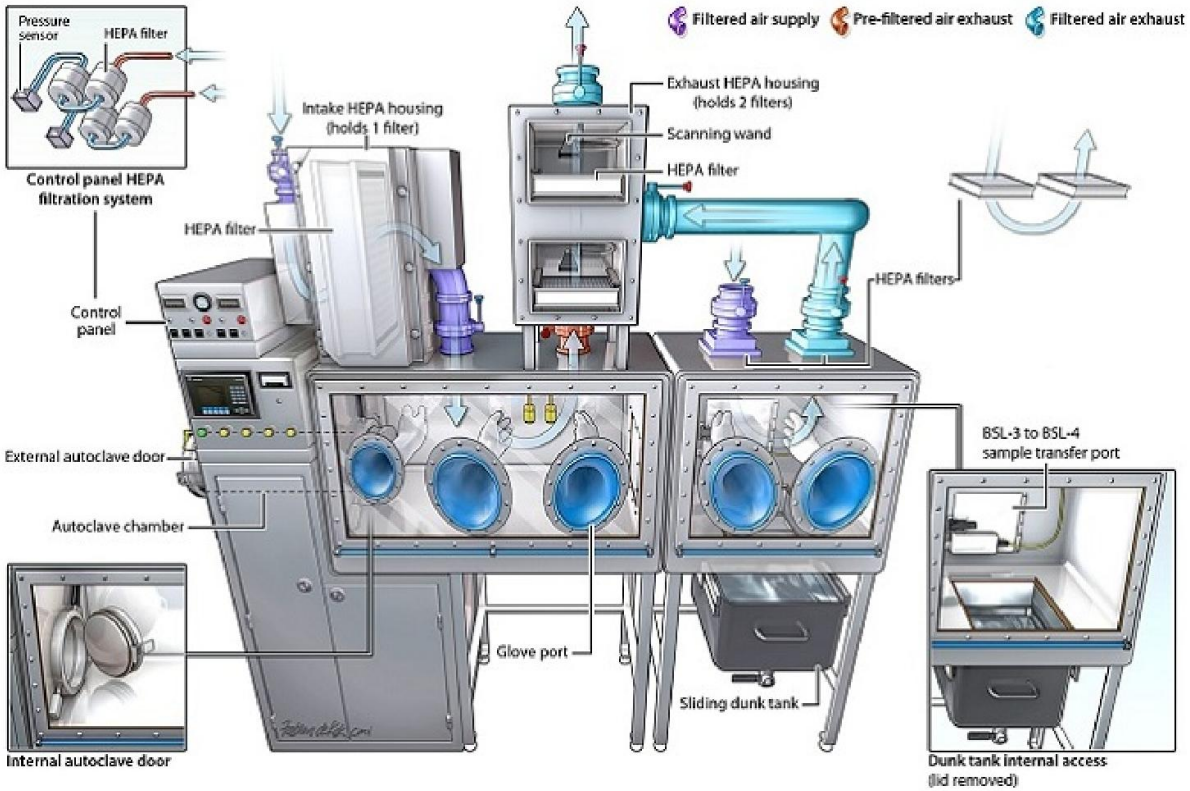


CLASS II BSC B2

- Protects person, product and environment.
- **Must be hard-ducted** **(can work with small amounts of chemicals)**
- Protects the person with 100 lfpm directional airflow into the cabinet.
- Protects the environment with HEPA filtered exhaust.
- Protects the product with HEPA filtered laminar airflow.
- 0% recirculation – 100% exhaust.

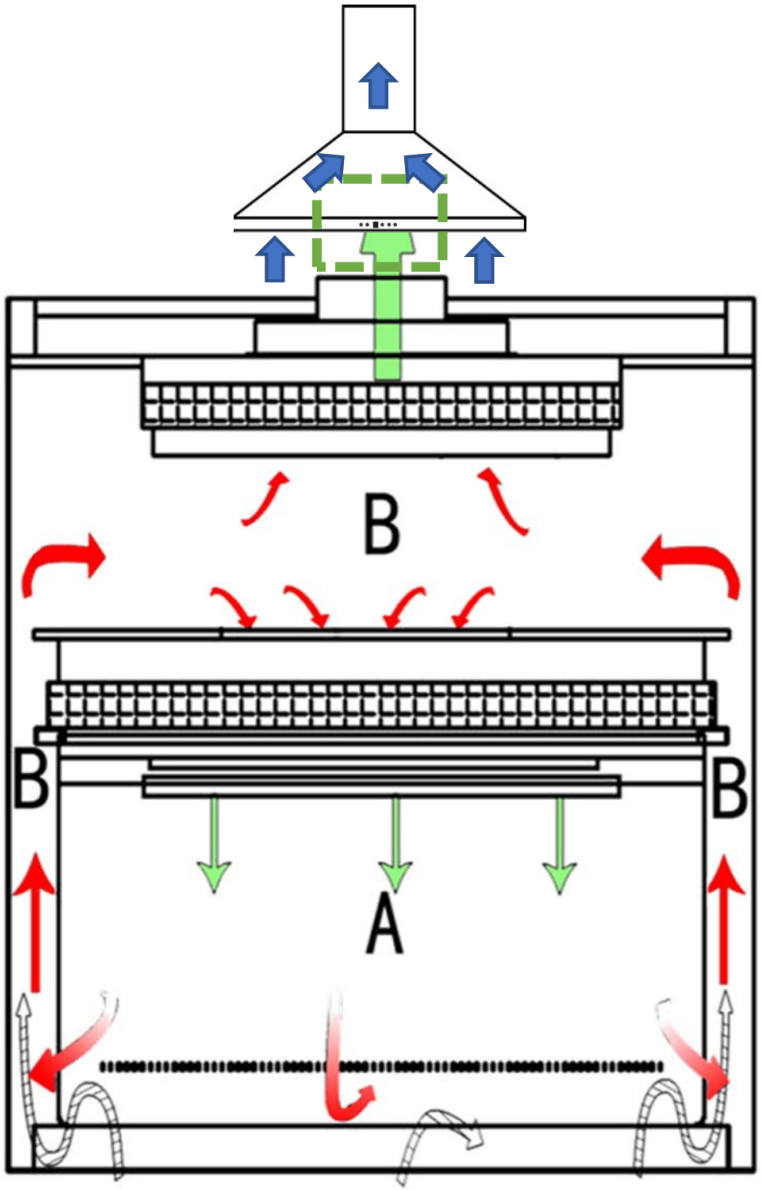


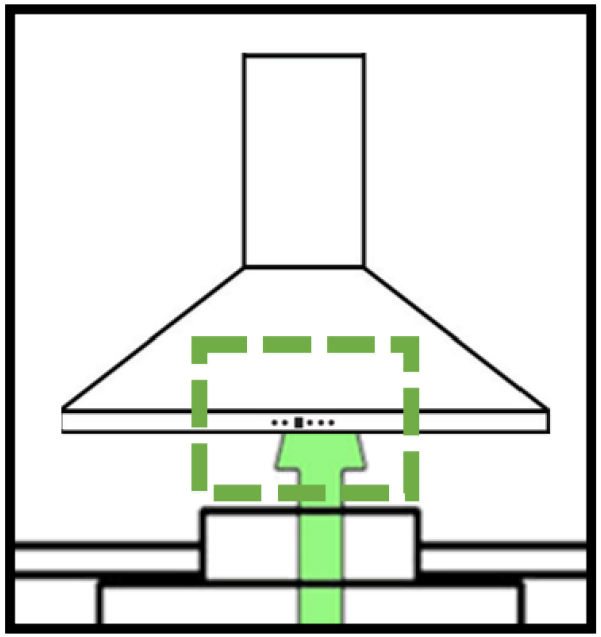
CLASS III BSC



- Protects person and environment.
- Must have single-HEPA filter supply – double-HEPA filtered exhaust.
- Must be sealed.
- Must have process for decontamination.
- May have double-door (interlocked) sample transfer port.

Thimble Connection





BENEFITS OF A THIMBLE CONNECTION

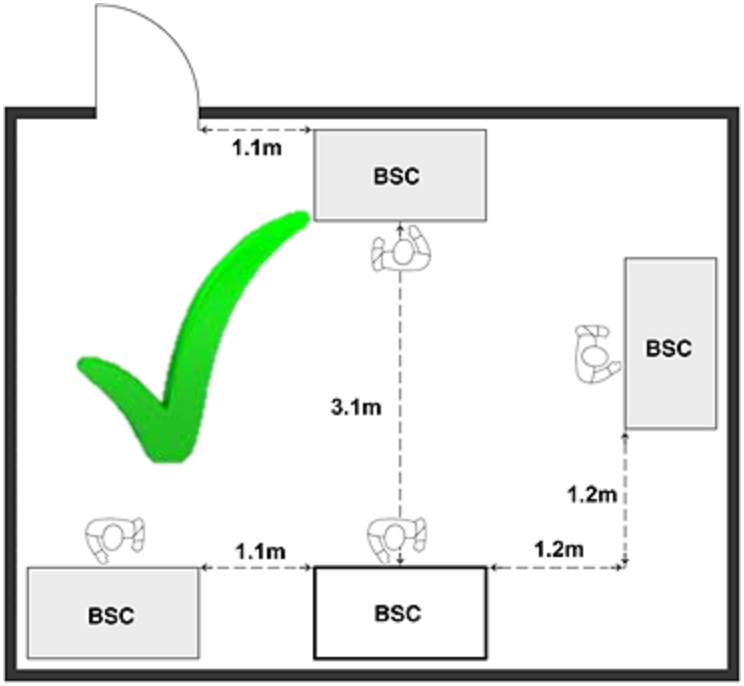
1. Saves $$ (Energy) – A2 for the benefit of a B2.
2. Minimize scents – smelly work.
3. You can shut off biosafety cabinet – and room continues to

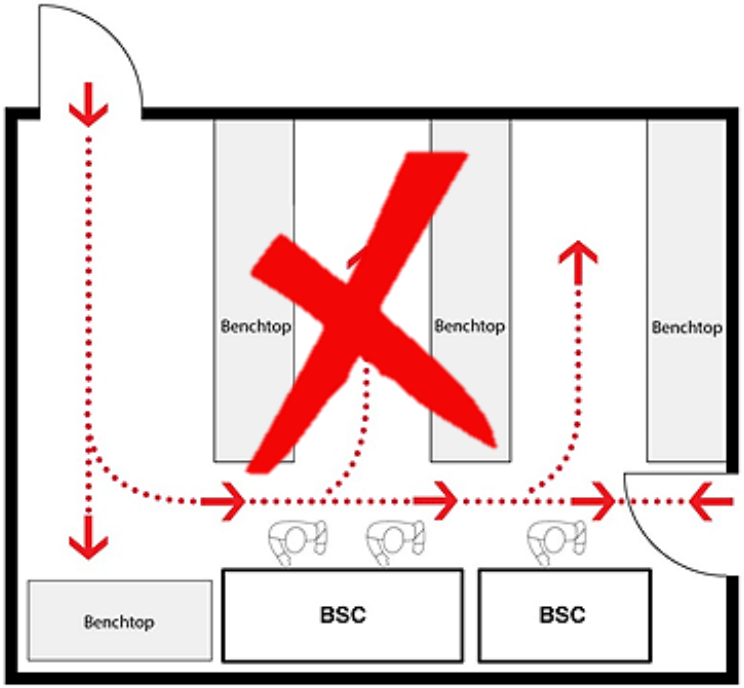
BSC Placement

- If cabinet is off – 4 minute start up is recommended followed by a complete wipe down before initial setup.
- Ultraviolet lamps are not recommended or needed for BSCs.
- Placement of the BSC is critical:

  1. 12 to 14 inch clearance above cabinet may be needed for adequate recirculation.
  2. Not placed near walkway – as the air curtain can be easily disrupted.
  3. Using smoke sticks, test to ensure air curtain is in tact – as supply air vents can also disrupt containment effectiveness of BSCs.

BSC Placement





Ultraviolet Light Considerations

The use of ultraviolet (UV) germicidal lamps is strongly discouraged due to their limited effectiveness at disinfecting the inside of BSCs.

- ✓ UV irradiation of the work area should only be used as a secondary method of disinfection in the cabinet. Never rely on UV irradiation alone to disinfect a contaminated work area.
- ✓ UV irradiation is ineffective if a **microorganism** is protected by dust, dirt, or organic matter. A liquid chemical disinfectant should be the primary method of cleaning and disinfecting the interior of a BSC
- ✓ UV irradiation does not penetrate into cracks or through the grilles of a BSC
- ✓ UV irradiation can cause deterioration of various materials, including certain plastics and tubing
- ✓ Never touch a UV bulb with bare hands as the natural oils from hands may leave a fingerprint and create dead space on the bulb's surface
- ✓ UV bulbs should be cleaned frequently with an appropriate disinfectant
- ✓ The UV lamp should be routinely tested with a UV meter to verify that the proper intensity (i.e., 40 $\mu W/cm^2$) is being delivered at the appropriate wavelength (i.e., 254 nm) in the centre of the work area.

BSC Certification

- Biosafety cabinets must be certified at the time of installation and annually from thereafter.

- Certification of the BSC includes:

  - Down flow velocity
  - Inflow velocity
  - Airflow smoke patterns
  - HEPA filter leak test
  - Cabinet integrity test
  - Light intensity test
  - Vibration test
  - Noise level test
  - UV lamp test

- Recertification should occur annually, if the BSC is moved, or following specific decontamination procedures.

thank you!