

General guideline for safety in teaching microbiology laboratory

Microbiology laboratories are categorized in different levels usually based on microbial agents that are isolated and studied in laboratory and also precautions required to work with them safely. These levels commonly referred as Biosafety level (BSL) or Pathogen/Protection level and range from the lowest at biosafety level 1 (BSL-1) to the highest at level 4 (BSL-4).

Biosafety level 1:

BSL-1 is well characterized with microbial agents that do not cause disease in Healthy person. e. g. non-pathogenic isolates of *Escherichia coli*, Staphylococci, Bacillus and some type of yeasts.

Research with these agents may be performed on standard open laboratory benches without the use of special containment equipment. It is not necessary for BSL-1 labs to be isolated from the general building but doors must keep closed when working. Windows are used for lightning and they also use for air conditioning. Frequent hand washing and using pathogen protection elements(PPE) are recommended.

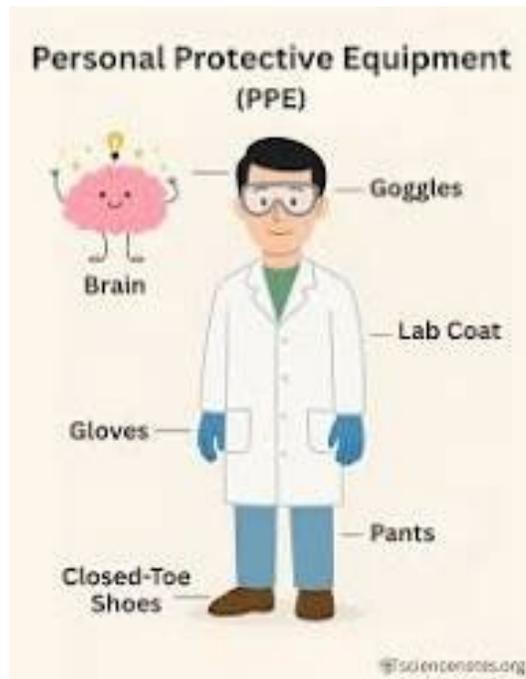


Figure 1, personal protection elements.

Biosafety level 2:

Various microbial agents that have moderate potential hazards and cause mild disease in healthy humans are categorized in BSL-2. Just like BSL-1, access to the laboratory is limited when work is

being conducted and more precaution is needed regarding splashes of microbial suspensions. Again laboratory could be in a general building but doors must keep closed when working. Windows are used for lightning and they also use for air conditioning. Frequent hand washing and using pathogen protection elements(PPE) are recommended. BSL-1 and BSL-2 laboratories usually used as teaching spaces for high schools and colleges.

Biosafety level 3:

BSL-3 involve microbial agents that could cause potentially lethal infections in human and they are able to transmit by inhalation route. e.g. *Francisella tularensis*, *Mycobacterium tuberculosis*, *Chlamydia psittaci*, SARS-Cov-2, *Coxiella burnetii*, *Rickettsia rickettsii*, *Yersinia pestis*. Vaccination or passive prophylaxis recommended for all laboratory personal(where available). Using Biosafety cabinet is recommended for experiments on all infectious materials. Solid-front protective clothing (i.e. gowns that tie in the back) is recommended instead of routine lab covers. It is recommended that laboratory placed in a separate building or separated from areas of the building with unrestricted traffic flow. Two sets of self-closing doors must be arranged at the laboratory entrance. Sealed windows recommended in BSL-3. Fully separate well equipped ventilation system is recommended that filtered the air before recirculation.



Figure 2, solid front laboratory cloth (Gown)

Biosafety level 4:

Infectious agents that transmitted by inhalation and cause fatal disease in healthy humans categorized in BSL-4. No vaccine or effective treatment is available for these agents. e.g. Marburg virus, Ebola virus, Lassa virus, and Crimean-Congo hemorrhagic fever. All experiments must run within a class III biosafety cabinet or in a class II biosafety cabinet by personnel wearing a positive pressure suit. All laboratory waste and infectious materials leaving the cabinet must be discard after

decontamination by autoclaving or suitable decontamination process. BSL-4 laboratories must be separated from surrounding building areas and equipped with specific air conditioning system. Airflow is tightly controlled and filtered before exhaustion or recirculation. The BSL-4 lab must be equipped with airlocks to minimize the possibility that aerosols leaked from the lab.



Figure 3, positive pressure protective suit that is recommended for BSL-4 facilities.



Figure 4, doors equipped with the airlock system arranged in entrance of a BSL-4 laboratory.

Persons that who work in a teaching medical microbiology laboratory may encounter with different type of hazards including; infectious agents, various chemical agents and even radioactive elements

in some cases. Therefore, all of the practitioners including student and also the laboratory staffs should consider different hazards and also know that how could do practices safely. All of the practitioners have to know the emergency protocols in case of accidental contamination with infectious agents and also hazardous chemicals.

General precautions for practitioners and staff:

- 1) Wash your hands with disinfectant soap when you arrive at the lab and again before you leave.
- 2) Absolutely no food, drinks, chewing gum, or smoking is allowed in the laboratory. Do not put anything in your mouth such as pencils, pens, labels, or fingers. Do not store food in areas where microorganisms are stored.
- 3) Using personal protective elements(PPE) such as lab coat, disposable gloves, mask and safety glasses is recommended. Leave protective clothing in the lab and do not wear it to other non-lab areas.
- 4) Do not leave unnecessary elements on the bench. Book or notebook, phone, purses, and coats should be placed in the cubbyholes by the front door of the lab.
- 5) Disinfect work areas before and after use with 70% ethanol or fresh 10% bleach. Laboratory equipment and work surfaces should be decontaminated with an appropriate disinfectant on a routine basis, and especially after spills, splashes, or other contamination.
- 6) Every practice with infectious agents is allowed only beside a flame(Bunsen burner or spirit lamp) or under safety cabinet. Do not open Petri dishes in any other conditions. Recap reagents, solution bottles, and bacterial suspensions.
- 7) Inoculating loops and needles should be flame sterilized with Bunsen burner, spirit lamp or electric loop sterilizer) before you lay them down.
- 8) Loops containing infectious material should be hold beside the flame before inoculation.
- 9) Vertexing and centrifugation of bacterial suspensions should be done in closed containers.
- 10) Turn off Bunsen burners when not is use. Long hair, shawl or scarf must be restrained if Bunsen burners are in use.
- 11) Never pipette by mouth. Use a pipetting aid or adjustable volume pipettors.
- 12) Autoclave grown plates and any infectious broth cultures to sterilize them before discarding.
- 13) Dispose of all contaminated solid waste material such as swab and cotton pads in a biohazard bag and autoclave it before discarding in the regular trash.
- 14) Familiarize yourself with the location of safety equipment in the lab (e.g., eye-wash station,
- 15) Report spills and accidents immediately to your instructor.

16) Report all injuries or accidents immediately to the instructor.

Three class of biological safety cabinet(BSC) are designed for laboratory use

Class I of BSC:

Class I cabinets provide low-level personnel protection but no environmental and product protection. In fact, the inward flow of air can contribute to contamination of samples. These BSCs are commonly used to enclose specific equipment (e.g. centrifuges) or procedures (e.g. aerating cultures) that potentially generate aerosols. BSCs of this class could use a duct as exhaust system or use unducted (recirculating exhaust back into the laboratory). This class of BSC usually are not equipped with HEPA(High efficiency particular air filters) and usually are not recommended for microbiology laboratories.

Class II of BSC:

Class II cabinets provide protection regarding the samples and also environmental since they are equipped with HEPA-filtered. Different types of BSC-II are available(A1, A2, B1, B2 and C1) and type A2 is most popular type.

In BSC_II several electrical blowers (fans) mounted in the cabinet to draw directional mass airflow around a user and into the cabin. The air is then drawn underneath from the holes or splits on the work surface and back up to the top of the cabinet where it passes through one or two series of the HEPA filters. Finally, air is exhausted through a HEPA filter, and depending on the Type of Class II BSC, the air is either liberated into the laboratory, to the cabinet or to a specific exhaust system. BSC_II generally use Laminar flow(the air moves with uniform velocity in one direction along parallel flow lines with little or no mixing). Therefore, BSC-II are commonly regarded as Laminar biosafety cabinets.

Class II Microbiology Safety Cabinet

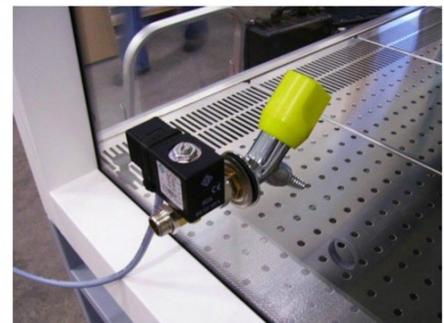
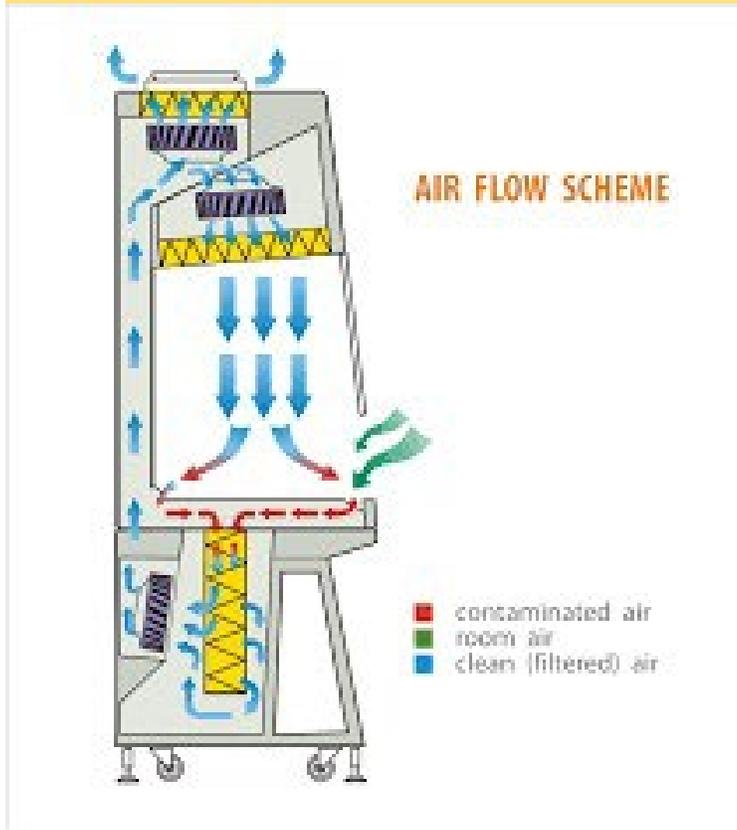


Figure 5, airflow diagram and shape of a Class II biosafety cabinet.

Class III of BSC or Glove box:

The Class III cabinet, generally only installed in maximum containment laboratories, is specifically designed for work with BSL-4 pathogenic agents, providing maximum protection. The enclosure is gas-tight, and all materials enter and leave through a dunk tank or double-door autoclave. Gloves attached to the front prevent direct contact with hazardous materials. These type of cabinets are usually custom-built.

Class III Microbiology Safety Cabinet (Glove Box)

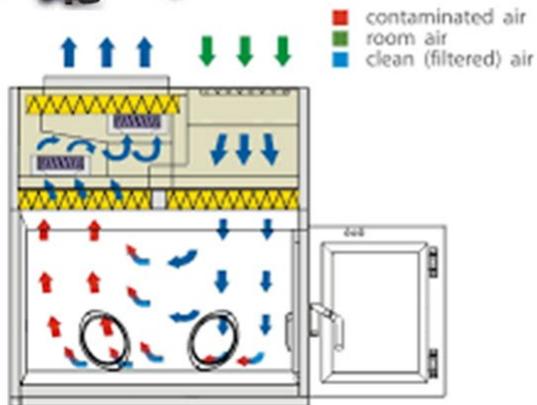


Figure 6, air flow and shape of a Glove box



Figure 7: a teaching laboratory that represent a BSL-I or BSL-2 properties. The floor, walls up to 1.5 m and bench surface are covered with a solid, stable and impenetrable materials such as granite or ceramic. Teflon covered sinks are recommended. Fully painted and anti-fouling faucets are implemented.

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