A Brief Overview of Biosafety Levels and Laboratory Practices in Clinical Laboratories: Containment and Control Measures

Abdul Waheed[1], Muhammad Roman [2], Zia Ashraf[1], Muhammad Mustafa Qamar[3], Iqra Bashir*[,4], Ghulam Khadija Bashir[5], Hasnain Javed[6], Muhammad Mudassar[1], Malaika Sajid[4] and Umer Ali[7]

[1]College of Allied Health Professionals, Government College University, Faisalabad (38000), Pakistan
[2]Microbiologist & Laboratory Manager Pathology Department, Surgimed Hospital, Lahore, Pakistan
[3]Department of Allied Health Sciences, University of Sargodha, Pakistan
[4]Institute of Microbiology (IOM), Government College University, Faisalabad 38000, Pakistan
[5]Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan
[6]Punjab AIDS Control Program complex, 6- Birdwood road, Lahore, Pakistan
[7]Department of Biological Sciences, Tennessee State University, Nashville, Tennessee 37209, United States of America
*Corresponding author: iqrabashir482@gmail.com

Abstract

The basic features of biosafety levels (BSLs) and laboratory procedures that are essential for guaranteeing secure operations in clinical laboratories are outlined in this chapter. It describes the agents handled, necessary procedures, and containment measures for each level of BSL classification, which ranges from minimal risk (BSL-1) to maximum confinement (BSL-4). To minimize the exposure risks, contamination, and outbreaks, the chapter highlights the significance of appropriate safety procedures, equipment use, and employee training. This chapter emphasizes the importance of structured biosafety practices in guaranteeing laboratory safety and protecting the public's health by discussing both general and specialized containment methods. However, the clinical laboratories should follow strict laboratory guidelines to reduce the chance of infection among laboratory personnel. Overall, it is significantly important to understand and to implement laboratory practices to protect laboratory personnel along with their working environment to minimize the risk and rate of infections, which ultimately promotes public health in a population.

Keywords: Biosafety levels, BSLs, Containment methods, Laboratory personnel, Public health

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Introduction

World Health Organization (WHO) defines biosafety as the containment principles, different technologies and procedures that are implemented in clinical laboratories to prevent the unintentional release of pathogens, their toxin or other harmful biohazard agents and their deliberate effect on human health, community, and environment (Resnik, 2024). Biosafety is a significant concern in laboratory settings, particularly in developing countries with a lack of proper standard operating procedures (SOPs), worldwide (Beeckman & Rüdelsheim, 2020; Novossiolova et al., 2021). The global fight against infectious diseases and exposures to working staff employed in clinical and biomedical laboratories where they come in contact with biological hazardous material that can cause laboratory-acquired infections (LAIs), depends heavily on biosafety during lab work and the movement of lab materials from one location to another (Aksoy et al., 2008). Handling blood or any other biological sample puts a lab worker at risk for unintentional exposure or damage. Workers at hospitals and laboratories in the public and commercial sectors are constantly exposed to known and undiscovered microorganisms, which puts them at risk for laboratory-acquired infections (Karamat et al., 2005; Nasim et al., 2010). Inadequate knowledge of biosafety-related issues leads to malpractice of the laboratory procedures and or improper handling of specimens during sample collection, processing, and disposal, which may increase exposure of laboratory professionals to pathogens. Lack of knowledge and a limited number of biosafety training programs about the handling of clinical samples and instruments are the main reasons of negligence among laboratory technicians in Pakistan (Mujeeb et al., 2003). These workers are continuously exposed to opportunistic pathogens or potentially pathogenic organisms, in addition to the possibility of infection transmission to others, as thousands of healthcare workers in developing countries, including Pakistan, sustain unintentional needle stick injuries every day (Maqbool, 2002; Habibullah & Afsar, 2007). Therefore, ensure biosafety in clinical laboratories to mitigate hazards of biological material such as pathogens and their genomic manipulation of the micro-organisms, drug-resistant pathogens, superbugs, creation of synthetic biological agents in the laboratory, and biological terrorism (Delany et al., 2011). This can be achieved by hiring highly trained and skilled laboratory personnel with high competencies in dealing with biological hazards and threats associated with these hazardous biological agents (Delany et

al., 2011). A number of different bacteria, viruses, and fungi cause laboratory-acquired infections (LAIs), so there should be special programmes and strategies for the identification of biological hazards, biological risk assessment, and their mitigation from the laboratory environment (Delany et al., 2011). The following are the competency domains for ensuring biosafety in laboratories (Table 1).

Table 1: Competency framework for ensuring biosafety in laboratory

	Potential hazards	**Description**	**References**
Domain-I	Biological materials	These biological materials, such as blood and others, are considered a potential hazard in laboratories.	(Yu et al., 2022)
	Research animals	Lab animals can be potentially harmful during handling in the laboratory.	(Alderman et al., 2018)
	Physical environment	Different actions in laboratory environments, such as spilling, etc., can be potentially harmful.	(Ta et al., 2019)
	Radiologic barriers	Radiological materials can be hazardous when used in conjunction with biological materials.	(Cornish et al., 2021)
	Chemical barriers	Chemicals in laboratories, even disinfectant, when handled not properly, can be potentially hazardous.	(Yu et al., 2022)
	Hazards control	Description	References
Domain-II	Personal protective equipment (PPE)	PPE such as gloves, lab coat, etc., plays an important role in preventing laboratory-acquired infections.	(Hrdinová et al., 2021)
	Primary Barriers	Some essential barriers include the techniques necessary to control hazards, such as working in a biosafety cabinet during work.	(Grane et al., 2017)
	Secondary barriers	Secondary barriers act as a backup safety measure, providing additional protection if primary barriers do not work.	(Joseph, 2021)
	Waste control and decontamination	Proper waste disposal and decontamination reduce the risk of infection.	(Cascardo et al., 2020)
	Administrative control	Description	References
Domain-III	Hazardous management	This includes things like implementing plans to control and minimize infection.	(Namasivayam et al., 2023)
	Regulatory compliance and guidelines	By following strict laboratory guidelines, the rate of infection can be minimized.	(Jirkof & Schmutz, 2019)
	Medical health surveillance	Monitoring the health status of personnel can help in the spread of infection.	(Wang et al., 2019)
	Risk management	It may involve establishing policies and protocols to reduce exposure to hazards.	(Handayani et al., 2024)
	Biosafety programs	It may involve various organizations and policies to work on biosafety.	(Gillum et al., 2024)
	Emergency preparedness and response	Description	References
Domain-IV	Emergencies and response to incidence	It may involve actions to reduce harm from various incidents, including spills and fires etc.	(Dowlati et al., 2021)
	Exposure, prevention, and the mitigation of biohazards	Techniques for spills and exposures to prevent biohazards.	(Miley, 2020)
	Exercise and drills for the purpose of a prompt response	By regularly practicing this, staff can develop a sense of ability to react efficiently in case of an emergency.	(Tang et al., 2024)

Biosafety Levels and Their Containment Facilities in Terms of Laboratory Biosafety

The biosafety levels are ranked in progressive order according to the protection they provide to humans, the environment, and the community. The standard microbiological procedures are followed in all laboratories. In addition to enhancing worker safety and environmental protection, certain microbiological protocols also reduce the danger of handling agents that require greater containment limits. There are four Biosafety levels in regard to containment in a clinical laboratory (Table 2).

Biosafety Level-1 (Minimal Risk)

BSL-I is designed to deal with micro-organisms that are not generally involved to cause disease in humans but pose a high risk to immunocompetent adult patients, the laboratory working staff, and its environment. These organisms are sometimes known as generally regarded as safe organisms (GRAS), such as *B. lichenformis, B. subtilis,* and the K-12 strain of *E. coli* (Nambisan, 2017).

Biosafety level I does not generally require to build in isolation from other laboratories. Usually, open bench tops are used for work, and conventional microbiological procedures are followed. This level doesn't require building special containment facilities due to the minimal possibility of risk posed by it. A researcher skilled with special microbiological procedures must monitor laboratory personnel in order to verify whether they have specialized training in operating the laboratory and experimentation being carried out in the lab or not. BSL-1 requires the following safety gear, established procedures, and containment facilities. Microorganisms like *E. coli* are handled in it (Bayot & Limaiem, 2019; Centers for Disease Control, 2020). BSL-I should be equipped with the following containment facilities:

Standard Microbiology Practices

The institutional regulations governing working access must be ensured by the supervisor of the biomedical laboratory. Proper hand

washing should be ensured before entering and leaving the lab. The application of makeup, handling of contact lenses with hands, the use of food, drinks, and smoking must be prohibited in the vicinity of the lab. Instead, this should be kept in a special cabinet specified for food storage. In addition to this, mouth pipetting must be prohibited and must be substituted with mechanical pipetting. A careful handling of needles, shattered glassware, scalpels, and pipettes, and safe and effective monitoring policies should be adopted. Sharp objects must always be handled with caution, including the following precautions such as syringe needles must not be handled in any way prior to disposal (Nasim et al., 2010). Disposable syringes and needles need to be stored carefully in easily accessible and puncture-resistant disposable receptacles.

Table 2: Overview of Biosafety levels (BSCS) for infectious agents in Clinical laboratory

Biosafety Levels	**BSL-I**	**BSL-II**	**BSL-III**	**BSL-IV**	**References**
Risk Groups	Risk Group-I	Risk Group-II	Risk Group-III	Risk Group-IV	
Basic description	● Without confinement to containment level ● Minimal risk of getting the disease	● Containment ● Moderate risk of disease ● The severity of the disease varies based on the pathogen	● High Containment ● Aerosol spread ● Serious potential of disease spread	● Max. Containment ● "Exotic" and indigenous risk-based Lethal agent ● Maximum risk agent	(World Health, 2004; Zrouri, 2022)
Pathogenic micro-organism	*E. Coli, B. subtilis, B. lichenformis*	This includes Human Immunodeficiency Virus (HIV) and Lyme disease etc.	*M. tuberculosis* *S.* Typhi	Marburg Virus and Ebola virus etc.	(Nambisan, 2017; Centre of Disease Control, 2020; Zrouri, 2022)
Pathogenicity level	Pathogens are not hazardous and prefer to work on a bench surface	Pathogens are moderately hazardous for laboratory workers and its environment	Indigenous or exotic agents posing serious aerosol transmission hazards, having the potential to spread potentially lethal disease agents	Highly lethal and exotic pathogens cause high levels of aerosol-based transmitted laboratory infections which are highly lethal	(World Health, 2004; Zrouri, 2022)
Containment practices	No containment is necessary, so open bench surface can be used	● Use of BSCs for the aerosol-generating pathogens ● Open bench surface ● Use of a proper waste management system	● Installation of HEPA filters to remove airborne pathogens ● Negative flow prevents backflow of the air from the lab. ● BSC	● Laboratories are isolated from other labs. ● Proper installation of the dedicated air waste system ● Air leaving labs is sterilized and purified ● Filtered air ● BSC-III in combination with BSC-II	(International Organization for Standardization, 2019).
Ventilation	None	None but a Controlled ventilation system and inward airflow is desirable	Inward airflow Controlled ventilating system HEPA filter air exhaust Double door entry Anteroom Anteroom with a shower Effluent treatment	Inward airflow Controlled ventilating system HEPA filter air exhaust Air lock system Air lock with shower Double door entry Anteroom Anteroom with a shower Effluent treatment	(Centre of Disease Control, 2020)
Autoclave requirements	None	None	Yes	Yes	(World Health, 2004; Zrouri, 2022)

In order to do this, work workplace should be cleaned with proper disinfectant. Necessary steps should be adopted to prevent the production of splashes and aerosol generation. Decontaminate all cultures, stocks, and other items in an efficient manner in an autoclave before their disposal. Furthermore, the bio-waste to be autoclaved must be packed in compliance with the federal standard. Bio-waste, which is to be discarded, must be transported in sealed containers outside the laboratory (Centers for Disease Control, 2020). A symbol named as biohazard symbol about the awareness of the infectious agents present outside the lab. The name of the pathogen under study and other accountable staff may be listed on the sign (Ta et al., 2018; Bayot & Limaiem, 2019). The lab supervisor is responsible for ensuring that laboratory personnel are well-trained in their duties, exposure prevention measures, and exposure evaluation protocols. Annual updates or extra training are required for staff members whenever procedures or policies change. An individual's vulnerability to infection, vaccination status, and capability to adopt precautionary measures in the lab affect the health status of the person. Therefore, information about immune competence and situations that may predispose people to infection should be given to all laboratory staff, especially women of childbearing age (Meechan & Potts, 2020).

Biosafety Level-II (Moderate Risk)

This includes the risk group- II pathogenic micro-organisms but are not likely to cause serious threats to individuals health, its community, livestock and the laboratory working environment such as *E. coli, Salmonella* spp, *Klebsiella* spp., *Penicillium marneffei, Blastomyces dermatitidis, Ascaris, Trypanosoma,* Adenovirus, Coronovirus and papilloma virus etc (Nambisan, 2017).

Biosafety Level II is an extension of BSL-I. It poses risk when working with biological agents or compounds that present modest risks to both the human and the its working environment. Special entry requirements should be made by the Principal investigatory (PI) and he should prepare some guidelines and procedures for the entrance to continue the operation of the laboratory. Laboratory staff should be highly skilled to handle biological agents and to carry out other laboratory procedures (Cornish et al., 2021). It is highly advisable to handle syringes with care. Use forceps, tongs, or a brush and dustpan to handle glassware. Every effort should be made to replace glasses with plastic. Apart from this, avoid creating aerosols or splashes by following all the procedures. An adequate disinfectant should be employed for cleaning work surfaces and spill management. All the culture, stocks, and potentially infectious biohazardous material must be autoclaved first. Use a sealed container for packaging the material that is essential to discard (Sarwar & Vijayan, 2021). Moreover, a symbol carrying a biohazards sign should be displayed in front of the laboratory to highlight the presence of infectious material inside the laboratory, as shown in Figure 1.

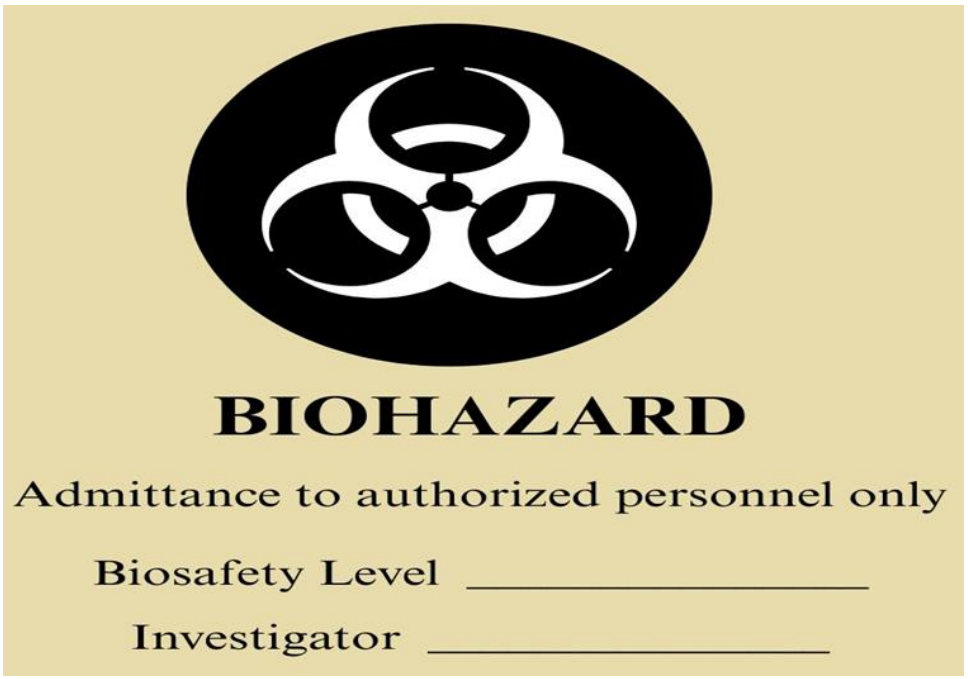

Fig. 1: A laboratory biohazard warning symbol for a laboratory door

Special Practices

Adhere to certain entry and exit protocols for entrance to the laboratory. Medical surveillance facility must be ensured at the door of the lab. It is essential to draft and implement a biosafety manual tailored to the laboratory demands (Sarwar & Vijayan, 2021).

Staff members who handle contagious items must control, disinfect, and clean up spills involving these materials. The supervisor of laboratory must be informed of any such incidences. Medical assessment, monitoring, and care should be given, and the proper documentation should be kept (Sarwar & Vijayan, 2021).

Use PPEs, remove them while entering non-laboratory areas. It is not advised to bring lab clothes home. Protective measures such as googles, face mask, facer shield and other splatter guards should be worn when pathogens are to be handled outside the BSC or splash is to be managed. Avoid exposure to biohazardous material by wearing gloves and other PPEs. Latex glove substitutes ought to be accessible. Wearing gloves outside of the lab is prohibited (Sarwar & Vijayan, 2021).

Secondary Barriers

BSCs need to be installed to ensure that variations in the room's exhaust and air supply don't impede normal operations. The best places for BSCs are away from doors, openable windows, busy lab spaces, and other potential airflow obstructions. Liquid disinfectant traps are advisable to safeguard vacuum lines and eyes wash stations are highly encouraged. Mechanical ventilation system should be installed. A Class II BSC HEPA-filtered exhaust air can be safely recycled back into the lab setting. Verification is required for the provisions that guarantee the appropriate functioning of the BSC and air flow (Toro, 2024; WHO, 2020).

Biosafety Level-3 (Serious Risk)

Biosafety level deals with highly serious pathogens and potential micro-organisms that pose serious risks and potential illnesses to laboratory individuals, but do not affect the community are the risk group such as *Francisella tularensis, Rickettsia* spp., *Histoplasma* spp., *Coccidiosis immitis*, Togavirus, Flavivirus, Pox Virus, West Nile Virus, and retroviruses, etc. (Nambisan, 2017).

A Biosafety Level 2 facility may provide the laboratory biosafety at a normal level. This is equipped with rooms where exhaust air is discharged outdoors, and the ventilation system (Sarwar & Vijayan, 2021). The Biological Safety Office should follow the PI policy (WHO, 2020).

Standard Microbiology Procedures

When working with cultures and specimens or conducting research, the Principal Investigator needs to not allow irrelevant person in the laboratory. Was hands before and after leaving the lab (WHO, 2020).

Moreover, in the work areas, avoid eatables, contact with items, and adopt mechanical pipetting. There are rules in place for handling sharp objects safely. Every step is taken with care to reduce the possibility of splashes or aerosols. Basic instructions are the same for all BSLs, such as culture disposal, spill management, contamination of work surface, use of PPEs, and transportation of the biohazard material (WHO, 2020; Zuo et al., 2024)

Special Practices

When studies are underway, the laboratory doors are kept closed, and the PI manages access, limiting entry to relevant person. Capillary tubes, slides, pipettes, scalpels, needles, and syringes should be handled with care. Anytime possible, glasses should be replaced with plastic wares. Safe technologies such as needless systems and re-sheathed syringes are utilized as necessary (Burnett et al., 2009). Moreover, there is no work done on the open bench in open containers. Using paper towels with a plastic backing to wipe down non-perforated work surfaces in BSC makes cleanup easier. After handling infectious materials, particularly following obvious spills, splashes, or other contamination, clean laboratory equipment and work surfaces with appropriate disinfectant. During collecting, handling, processing, storage, transportation, or shipment, cultures, tissues, bodily fluid specimens, or wastes are put in a container that stops leaks (Burnett et al., 2009).

Primary Physical Barriers

When in the laboratory, one should wear protective gear such lab coat, solid-front. Outside of the laboratory, protective gear is not worn. Before being laundered, clothes are disinfected. When clothing becomes obviously contaminated, it is changed. It is advised to often change gloves and wash your hands after handling contaminated equipment and materials. Never use disposable gloves (Ta et al., 2018).

All infectious material manipulations, necropsies of infected animals, tissue or fluid harvesting from infected animals or embryonic eggs, etc., are carried out in in BSC-II and BSC-III. When in rooms with diseased animals, protective gear is worn on the face and respiratory system (WHO, 2020).

Secondary Barriers

The lab has restricted access and is situated apart from parts of the building where circulation may flow freely. Areas handling BSL3 agents have interior surfaces made for simple cleaning and disinfection, including the walls, floors, and ceilings. Any seams that exist need to be sealed. Smooth surfaces, liquid-impermeable walls, and floors that are impervious to the chemicals and disinfectants typically used in laboratories are ideal (Ta et al., 2018).

Monolithic, non-slip floors are ideal. Coved floor coverings should be taken into consideration. Walls, ceilings, and flooring all have sealed penetrations. It is possible to seal gaps between doors and frames, as well as surrounding ducts, to make decontamination easier (Burnett et al., 2009). Bench tops should be properly cleaned with ethanol (Ta et al., 2018).

A decontamination process is available and used in the facility for all laboratory wastes, ideally within the laboratory. It is important to think about ways to decontaminate equipment. Waste must be appropriately sealed while leaving the lab and should never be carried into public hallways. Room supply louvers, doors, and heavily trafficked laboratory areas must all be kept away from BSCs (WHO, 2020).

The exhaust from outside must either be HEPA-filtered or distributed away from occupied spaces and air intakes (Smith et al., 2006; Bannister et al., 2009). The airflow into the laboratory must be checked for proper direction by laboratory staff. It is advised that the laboratory entrance be equipped with a visual monitoring system that verifies and indicates directed inward airflow. To avoid the lab becoming overly pressurized, the installation of an HVAC control system should be taken into consideration. Employees should be alerted to HVAC system failures by means of audible alarms. Recirculating HEPA-filtered exhaust air into the laboratory from a Class II BSC is possible if the cabinet is approved and tested at least once a year (Centre for Disease Control, 2020; Xia & Yuan, 2022).

Following that, Class II BSC exhaust air must be released outside (Zhang et al., 2017). Class III BSCs ought to be attached straight to the exhaust system when in use. Equipment that could create aerosols, such as continuous flow centrifuges, is found in devices that exhaust air via HEPA filters before releasing it into the laboratory. The HEPA systems must be inspected at least once a year (Xia & Yuan, 2022).

Summary of the recommended infectious agents in different BSLs is given in Table 2.

Biosafety Level-4 (Maximum Risk)

These are highly exotic and indigenous pathogens belonging to the risk group IV, found to cause life-threatening and serious diseases among individuals and their community, and can be readily transmitted from person to person. They lack effective treatment availability, such as Marburg virus, Ebola Virus, Central European encephalitis, and Herpes virus, etc. (Nambisan, 2017).

BSL-IV is required while handling highly lethal and exotic pathogens which cause a high risk of transmission of aerosols and have the potential to put laboratory staff, the environment, and the community at high risk. This may cause a potentially life-threatening disease with an unknown cause for which there is no immunization or vaccination programme is available at that time (Peters, 2018). Highly skilled laboratory staff should handle extremely dangerous infectious pathogens; laboratory personnel need to be properly and specifically trained. Staff members working in laboratories must be proficient in handling agents and processes that need BSL-4 containment (Richmond & McKinney, 2009; Xia & Yuan, 2022).

Two BSL-4 laboratory models are available, one is a cabinet laboratory which uses BSC-III, and connection of the cabinet exhaust to an independent building exhaust air system is required. The other one is a suit laboratory, where protective suits with positive pressure must be worn by personnel. BSL-4 and suit laboratories serve to prevent the dissemination of pathogens to the surrounding environment. The

institutional rules governing laboratory access must be upheld by the lab supervisor (Centre for Disease Control, 2020).

Avoid touching contact lenses, using makeup, eating, and drinking in open spaces while working in BSL-IV. Keep your food stored in a refrigerator specifically designed for keeping food instead of taking it into the laboratory. It is necessary to employ mechanical pipetting devices. Disposable needles should be disposed of near the containers designed to withstand punctures (Ulanday & Lamata-Porras, 2025).

After work is finished and any potentially infectious material has spilled or splashed onto surfaces, disinfect them using a suitable disinfectant. Use an effective, safe, and effective process to decontaminate all wastes before removing them from the laboratory. A symbol representing biohazard material must be displayed at the entrance of the biomedical laboratory. Posting agent information should adhere to the institutional policy (Yabut, 2024; Ulanday & Lamata-Porras, 2025).

Annual updates or additional training must be provided to staff whenever procedures or policies change. One's ability to obtain vaccinations or preventative measures, as well as their vulnerability to infection, might be influenced by their personal health status. Thus, all laboratory staff members should get information, especially women who are of childbearing age (Hall et al., 2025).

Only authorized person should enter the lab for their specific purpose. Entrance to the lab should be restricted with a secure lock. Logbook should be maintained. Entrance or exit should be restricted except through the clothing change room or shower room, other than in an emergency situation. Take a shower soon after removing lab clothing, such as overalls. Decontaminate all the material before leaving the lab in an autoclave. After decontaminating the lab environment, all the staff and students can enter or leave the lab without any restriction (Hall et al., 2025; Vourtsis et al., 2025).

Laboratory staff must be skilled with specific training related to medical surveillance, immunization of the laboratory personnel, and the handling of potentially lethal agents to be handled in the laboratory for their safety. It is necessary to set up a system for reporting and recording laboratory exposures, accidents, and employee absences as well as for medical surveillance of possible illnesses linked to the lab. Biological agents must be treated in sealed containers (Zuo et al., 2024; Vourtsis et al., 2025).

Equipment or materials that may suffer damage by steam or high temperatures must be sterilized using an effective and validated method, such as a gaseous or vapor approach, in an airlock or chamber designed for this purpose. Any emergency situation must be addressed immediately and managed in compliance with the laboratory biosafety manual's recommendations. It is necessary to notify the laboratory supervisor, the institution's management, and the relevant authorities (Kraus & Mirazimi, 2013).

Primary Barriers

Decontaminating items leaving the Class III BSC requires the provision of double-door, pass-through autoclaves. A HEPA filter is essential to be installed on the inlet of the air supply of BSC-III. Two HEPA filters are adjusted on the output in series. Gas-tight dampers are adjusted on the inlet and outlet of the air supply of the filters for the purification of air vapours before exit (Abad et al., 2024; Teotia et al., 2024).

Remove all sharp edges from cabinet finishes to lessen the chance of glove rips and injuries. The BSC-III gloves should be replaced every year, and all equipment must be clear of sharp edges to prevent damage. When possible, the cabinet should be built so that its mechanical systems, such as the centrifuges, incubators, and refrigerators, can be maintained and repaired from the outside. Physical containment strategies should be employed in BSC-III in order to manipulate large number of infectious pathogens wherever possible. Use centrifuge safety cups or sealed rotor heads to centrifuge such materials inside the cabinet. Every year at the very least, the Class III cabinet needs to be certified (Toro, 2024).

Air that can generate aerosols must be purified before its release into the laboratory. This must be exhausted through HEPA filtration in primary barrier devices. Annual testing and replacement of these HEPA filters are recommended (Toro, 2024; Zuo et al., 2024).

Control Measures

To reduce the dangers of exposure and environmental contamination, containment techniques are crucial. To ensure worker and public safety, for instance, HEPA filtration in BSL-3 and BSL-4 labs efficiently captures airborne infections should be installed (Toro, 2024). In addition to protecting their employees, clinical labs uphold integrity and trust in their work by putting these controls in place.

Conclusion

Biosafety is a significant concern in laboratory settings, particularly in developing countries with a lack of proper standard operating procedures (SOPs), worldwide. The global fight against pathogens and their related infectious diseases and exposures to working staff employed in clinical and biomedical laboratories, where they come in contact with hazardous biological material. Clinical labs run safely and responsibly by virtue of biosafety standards and associated procedures. These precautions, which range from regular hand washing to sophisticated containment procedures, can reduce hazards while facilitating vital medical developments. By adhering to these guidelines, laboratories can carry out their vital tasks without endangering worker safety.

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