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Government of India
Ministry of Health

BIOSAFETY MANUAL
FOR
PUBLIC HEALTH LABORATORIES

National Centre for Disease Control
22 Sham Nath Marg
New Delhi – 110054

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Directorate General of Health Services
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Chapter 1
General Principles of Biosafety

1.1. What is Biosafety?

"Laboratory bio-safety" is the term used to describe the containment principles, technologies and practices that are implemented to prevent unintentional exposure to pathogens and toxins, or their accidental release.

"Laboratory biosecurity" refers to institutional and personal security measures designed to prevent the loss, theft, misuse, diversion or intentional release of pathogens and toxins.

Bio-safety protection is to protect laboratory workers, clinical specimens and the environment. Diagnostic and health-care laboratories (public health, clinical or hospital-based) must all be designed for at least Bio-safety Level 2 or above if required. As no laboratory has complete control over the specimens it receives, standard precautions should always be adopted and practiced.

The basic objective of a biosafety program is the containment of potentially harmful biological agents. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents. The use of vaccines may provide an increased level of personal protection. The term "containment" is used in describing safe methods, facilities and equipment for managing infectious materials in the laboratory environment where they are being handled or maintained. The appropriate combination of the elements of containment required in a laboratory is determined on the basis of the risk assessment of the work to be done with a specific agent.

1.2 Elements of containment

- Persons working in the laboratory must follow the basic elements of containment which include:
  - Strict adherence to standard microbiological practices and techniques.
  - Awareness of potential hazards among persons working with infectious agents or potentially infected materials
  - Must be trained and proficient in the practices and techniques required for handling hazardous material safely.

- Each laboratory should develop or adopt a biosafety or operations manual that identifies the hazards that will or may be encountered, and that specifies practices and procedures designed to minimize or eliminate exposures to these hazards. When standard laboratory practices are not sufficient to control the hazards associated with a particular agent or laboratory procedure, additional measures may be needed which include:
  - Safety equipment – These are the *primary barriers* and include use of appropriate biosafety cabinets, safe centrifuges and appropriate Personal Protective Equipment (PPE) such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles as per the risk assessment.
  - Appropriate facility design and engineering features – These are the *Secondary barriers* and provide protection to the laboratory workers, to persons outside the laboratory, and persons or animals in the community from infectious agents that may be accidentally released in the laboratory.
  - Management practices must supplement laboratory personnel, safety practices, and techniques.
- The recommended secondary barrier(s) will depend on the risk of transmission of specific agents. When the risk of infection by exposure to an infectious aerosol is present, higher levels of primary containment and multiple secondary barriers may become necessary to prevent infectious agents from escaping into the environment. Such design features include:
  - Specialized ventilation systems to ensure directional airflow
  - Air treatment systems to decontaminate or remove agents from exhaust air
  - Controlled access zones
  - Airlocks at laboratory entrances, or
  - Separate buildings or modules to isolate the laboratory

1.3 Biosafety Levels

Four Biosafety Levels (BSLs) are described based on combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the type of procedures performed and documented or suspected routes of transmission of infectious agents. BSL-1 are basic teaching laboratories, BSL-2 are diagnostic services and research laboratories, BSL-3 are high containment special diagnostic services laboratories and BSL-4 are maximum containment laboratories to handle dangerous pathogens. Laboratory biosafety manual (3rd Ed), WHO, describes relative hazards of infective microorganisms by risk groups for laboratory work (Table 1.1). The classification of microbiological agents is based on their association with, and resulting severity of, disease in humans. The risk group of an agent should be one factor considered in association with mode

of transmission, procedural protocols, experience of staff, and other factors in determining the BSL in which the work will be conducted (Table 1.2).

The recommended biosafety level(s) for the organisms represent those conditions under which the agent ordinarily can be safely handled. The laboratory in charge is specifically and primarily responsible for assessing the risks and applying the appropriate biosafety levels. The institution's Biosafety Officer (BSO) and Institutional Biosafety Committee (IBC) can be of great assistance in performing and reviewing the required risk assessment.

Table 1.1. Classification of infective microorganisms by risk group (WHO Laboratory Biosafety Manual, 3rd Ed, 2004)

Risk Group 1 (no or low individual and community risk) A microorganism that is 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 that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

Table 1.2. Relation of risk groups to biosafety levels, practices and equipment (WHO Laboratory Biosafety Manual, 3rd Ed, 2004)

Risk group	Biosafety level (BSL)	Laboratory type	Laboratory practices	Safety equipment
1	BSL 1	Basic teaching, research	Good Microbiological Techniques (GMT)	None; open bench work
2	BSL 2	Primary health services; diagnostic services; research	GMT plus protective clothing, biohazard sign	Open bench plus Biological Safety Cabinet (BSC) for potential aerosols
3	BSL 3	Special diagnostic services, research	As Level 2 plus special clothing, controlled access, directional airflow	BSC and/or primary devices for all activities
4	BSL 4	Dangerous pathogen units	As Level 3 plus airlock entry, shower exit, special waste disposal	Class III BSC, or positive pressure suits in conjunction with Class II BSCs, double ended autoclave (through the wall), filtered air

1.4 Risk assessment

Risk assessment is a process used to identify the hazardous characteristics of a known infectious or potentially infectious agent or material, the activities that can result in a person's exposure to an agent, the likelihood that such exposure will cause a Laboratory Associated Infection (LAI), and the probable consequences of such an infection. The information identified by risk assessment will provide a guide for the selection of appropriate biosafety levels and microbiological practices, safety equipment, and facility safeguards that can prevent LAIs.

Following factors should be considered for conducting risk assessment:

- Risk group of the microbiological agent (Table 1)
- Pathogenicity of the agent and infectious dose
- Potential outcome of exposure
- Natural route of infection
- Other routes of infection, resulting from laboratory manipulations

    - Direct contact – skin, mucosa or eye
    - Parenteral – inoculation (needle or contaminated sharp), bite from infected vectors
    - Airborne - inhalation
    - Ingestion
    - Handling of laboratory animals
- Stability of the agent in the environment
- Concentration of the agent and volume of concentrated material to be manipulated
- Presence of a suitable host (human or animal)
- Information available from animal studies and reports of laboratory-acquired infections or clinical reports
- Laboratory activity planned (sonication, aerosolization, centrifugation, etc.)
- Any genetic manipulation of the organism that may extend the host range of the agent or alter the agent's sensitivity to known, effective treatment regimens
- Local availability of effective prophylaxis or therapeutic interventions
- Endemicity of the organism – Non indigenous agents are of special concern because of their potential to introduce risk of transmission.

Specimens for which there is limited information

In situations when the information is insufficient to perform an appropriate risk assessment, for example, with clinical specimens or epidemiological specimens collected in the field, cautious approach to specimen manipulation should be followed as follows:

- Follow Standard precautions
- Follow barrier protections and PPE (gloves, gowns, eye protection), whenever specimens are obtained from patients.
- Follow at least Biosafety Level 2 practices and procedures for handling specimens.
- Follow national and/or international rules and regulations for transport of specimens
- Some information may be available to assist in determining the risk of handling these specimens:
    - Medical data on the patient
    - Epidemiological data (morbidity and mortality data, suspected route of transmission, other outbreak investigation data)
    - Information on the geographical origin of the specimen

In the case of outbreaks of disease of unknown etiology, appropriate ad hoc guidelines may be generated to indicate how specimens should be consigned for shipment and the biosafety level at which they should be analysed.

1.5 Practices followed at various biosafety levels

Biosafety Level 1 practices, safety equipment, and facility design and construction are appropriate for undergraduate and secondary educational training and teaching laboratories, and for other laboratories in which work is done with defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans e.g. *Bacillus subtilis.* Many agents not ordinarily associated with disease in humans are, however, opportunistic pathogens and may cause infection in the young, the aged, and immunodeficient or immunosuppressed individuals. Vaccine strains that have undergone multiple *in vivo* passages should not be considered avirulent simply because they are vaccine strains.

BSL-1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for hand washing.

Biosafety Level 2 practices, equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, and other laboratories in which work is done with the broad spectrum of indigenous moderate-risk agents that are present in the community and associated with human disease of varying severity. With good microbiological techniques, these agents can be used safely in activities conducted on the open bench, provided the potential for producing splashes or aerosols is low e.g. work with the *Salmonella*, Hepatitis B virus, HIV, *Toxoplasma* etc. BSL-2 is appropriate when work is done with any human-derived blood, body fluids, tissues, or primary human cell lines where the presence of an infectious agent may be unknown.

Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials. Extreme caution should be taken with contaminated needles or sharp instruments. Even though organisms routinely manipulated at BSL-2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment, or in devices such as a BSC or safety centrifuge cups. Personal protective equipment should be used as appropriate, such as splash shields, face protection, gowns, and gloves.

Secondary barriers, such as hand washing sinks and waste decontamination facilities, must be available to reduce potential environmental contamination.

Biosafety Level 3 practices, safety equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents with a potential for respiratory transmission, and which

may cause serious and potentially lethal infection e.g. CCHF, Influenza viruses etc. Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols. At BSL-3, more emphasis is placed on primary and secondary barriers to protect personnel in contiguous areas, the community, and the environment from exposure to potentially infectious aerosols. Secondary barriers for this level include controlled access to the laboratory and ventilation requirements that minimize the release of infectious aerosols from the laboratory.

Biosafety Level 4 practices, safety equipment, and facility design and construction are applicable for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy. Agents with a close or identical antigenic relationship to BSL-4 agents also should be handled at this level. When sufficient data are obtained, work with these agents may continue at this level or at a lower level e.g. Ebola & Marburg viruses etc.

The primary hazards to personnel working with BSL-4 agents are respiratory exposure to infectious aerosols, mucous membrane or broken skin exposure to infectious droplets, and autoinoculation. All manipulations of potentially infectious diagnostic materials, isolates, and naturally or experimentally infected animals, pose a high risk of exposure and infection to laboratory personnel, the community, and the environment.

The laboratory worker's complete isolation from aerosolized infectious materials is accomplished primarily by working in a Class III BSC or in a full-body, air-supplied positive-pressure personnel suit. The BSL-4 facility itself is generally a separate building or completely isolated zone with complex, specialized ventilation requirements and waste management systems to prevent release of viable agents to the environment.

Summary of biosafety level requirements are given in Table 1.3. The laboratory director is specifically and primarily responsible for the safe operation of the laboratory. His/her knowledge and judgment are critical in assessing risks and appropriately applying these recommendations. The recommended biosafety level represents those conditions under which the agent can ordinarily be safely handled. Special characteristics of the agents used, the training and experience of personnel, procedures being conducted and the nature or function of the laboratory may further influence the laboratory director in applying these recommendations.

Table 1.3. Summary of biosafety level requirements (WHO Laboratory Biosafety Manual, 3rd Ed, 2004)

	Biosafety level			
	1	**2**	**3**	**4**
Isolation of laboratory	No	No	Yes	Yes
Room sealable for decontamination	No	No	Yes	Yes
Ventilation:				
• Inward airflow	No	Desirable	Yes	Yes
• Controlled ventilating system	No	Desirable	Yes	Yes
• HEPA filtered air exhaust	No	No	Yes/No	Yes
Double door entry	No	No	Yes	Yes
Airlock	No	No	No	Yes
Airlock with shower	No	No	No	Yes
Anteroom	No	No	Yes	-
Anteroom with shower	No	No	Yes/No**	No
Effluent treatment	No	No	Yes/No**	Yes
Autoclave				
• On site	No	Desirable	Yes	Yes
• In laboratory room	No	No	Desirable	Yes
• Double-ended	No	No	Desirable	Yes
Biological safety cabinets	No	Desirable	Yes	Yes
Personnel safety monitoring capability***	No	No	Desirable	Yes

**Depending on agents used in laboratory

***Glass windows, closed circuit television, two-way communication

Chapter 2
Biosafety Cabinets

Biological Safety Cabinets (BSCs) is an important piece of safety equipment in which manipulations of infectious microorganisms are performed. These are designed to protect the operator, the laboratory environment and work materials from exposure to infectious aerosols and splashes that may be generated when manipulating materials containing infectious agents, such as primary cultures, stocks and diagnostic specimens. Aerosol particles are created by any activity that imparts energy into a liquid or semi liquid material, such as shaking, pouring, stirring or dropping liquid onto a surface or into another liquid. BSCs are highly effective in reducing laboratory-acquired infections and cross-contaminations of cultures due to aerosol exposures & also protect the environment.

An essential component of the BSCs is the high-efficiency particulate air (HEPA) filter in the exhaust and/or supply systems which remove microscopic contaminants from the air. The HEPA filter traps 99.97% of particles of 0.3 µm in diameter. This enables the HEPA filter to effectively trap all known infectious agents and ensure that only microbe-free exhaust air is discharged from the cabinet.

A BSC must be routinely inspected and tested following strict protocols to verify that it is working properly. This process is referred to as certification of the cabinet and should be performed annually. A properly certified and operational BSC is an effective engineering control that must be used in concert with the appropriate practices, procedures and other administrative controls to further reduce the risk of exposure to potentially infectious microorganisms.

2.1 Types of Biosafety cabinets (BSCs)

2.1.1. Class I biological safety cabinet

The Class I BSC provides personnel and environmental protection, but no product protection. Unfiltered room air is drawn in through the front opening (for use by the operator's arms to reach the work surface inside the cabinet), it passes over the work surface and is discharged from the cabinet through the exhaust duct. The directional flow of air whisks aerosol particles that may be generated on the work surface away from the laboratory worker and into the exhaust duct. The air from the cabinet is exhausted through a **HEPA filter** to the outside**.** (Fig 2.1)

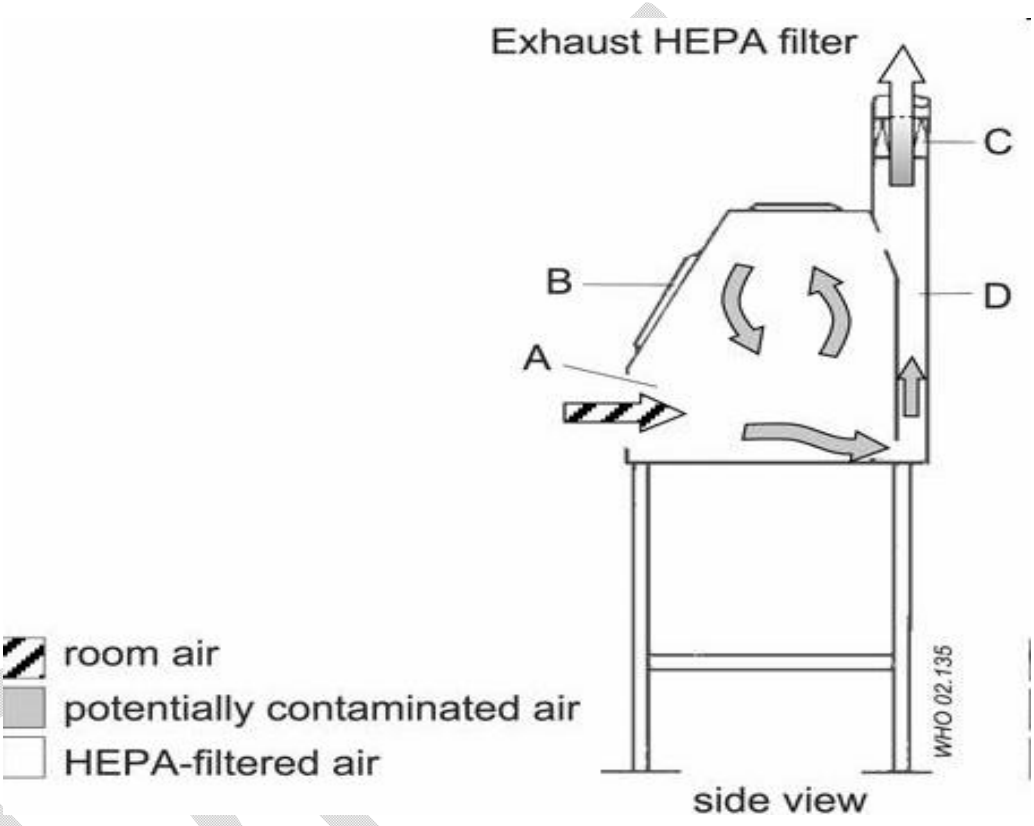


Fig: 2.1 Schematic representation of a Class I biological safety cabinet. A: front opening, B: sash, C: exhaust HEPA filter, D: exhaust plenum. (BMBL 5th edition)

2.1.2. Class II biological safety cabinets

Class II BSCs, differ from Class I BSCs by allowing only air from a HEPA-filtered (sterile) supply to flow over the work surface, therefore providing product protection. Class II BSCs are partial barrier systems that rely on the directional movement of air to provide containment. Airflow is drawn into the front grille of the cabinet, providing personnel protection. In addition, the downward flow of HEPA-filtered air provides product protection by minimizing the chance of cross-contamination across the work surface of the cabinet. Because cabinet exhaust air is

passed through a certified HEPA filter, it is particulate-free (environmental protection), and may be recirculated to the laboratory (Type A1 and A2 BSCs) or discharged from the building via a canopy or "thimble" connected to the building exhaust (Fig. 1.4.).

As the air curtain is disrupted (e.g., movement of materials in and out of a cabinet, rapid or sweeping movement of the arms) the potential for contaminant release into the laboratory work environment is increased, as is the risk of product contamination.

The Class II BSCs (Types A1, A2, B1 and B2) provide personnel, environmental and product protection.

Class II Type A1 Cabinets (Fig. 2.2)

- An internal fan (Figure 2.2) draws sufficient room air through the front grille to maintain a minimum calculated or measured average inflow velocity of at least 75 ft/min at the face opening of the cabinet. The supply air flows through a HEPA filter and provides particulate-free air to the work surface. Airflow provided in this manner reduces turbulence in the work zone and minimizes the potential for cross-contamination.
- The downward moving air "splits" as it approaches the work surface; the fan draws part of the air to the front grille and the remainder to the rear grille. Although there are variations among different cabinets, this split generally occurs about halfway between the front and rear grilles and two to six inches above the work surface.
- The air is drawn through the front and rear grilles by a fan pushed into the space between the supply and exhaust filters. Due to the relative size of these two filters, approximately 30% of the air passes through the exhaust HEPA filter and 70% recirculates through the supply HEPA filter back into the work zone of the cabinet. Most Class II, Type A1 and A2 cabinets have dampers to modulate this division of airflow.

Class II, Type A2 Cabinets (Fig. 2.3)

- A Class II A2 cabinet must have a minimum inflow velocity of 100 ft/min, allowing it to be used for volatile chemicals adjunct to microbiological studies, if properly exhausted outdoors via a canopy exhaust connection. In other respects, the specifications are identical to those of a Type A1.

Exhausted A1 and A2 BSCs: It is possible to exhaust the air from a Type A1 or A2 cabinet outside of the building. However, it must be done in a manner that does not alter the balance of the cabinet exhaust system, thereby disturbing the internal cabinet airflow. The proper method of connecting a Type A1 or A2 cabinet to the building exhaust system is through use of a canopy hood, which provides a small opening or air gap (usually 1 inch) around the cabinet exhaust filter housing (Fig. 2.4.). The airflow of the building exhaust must be sufficient to maintain the flow of room air into the gap between the canopy unit and the filter housing. The

canopy must be removable or be designed to allow for operational testing of the cabinet. (See Section VI.)

Note: Class II Type A1 or A2 cabinets should never be hard-ducted to the building exhaust system. Fluctuations in air volume and pressure that are common to all building exhaust systems sometimes make it difficult to match the airflow requirements of the cabinet.

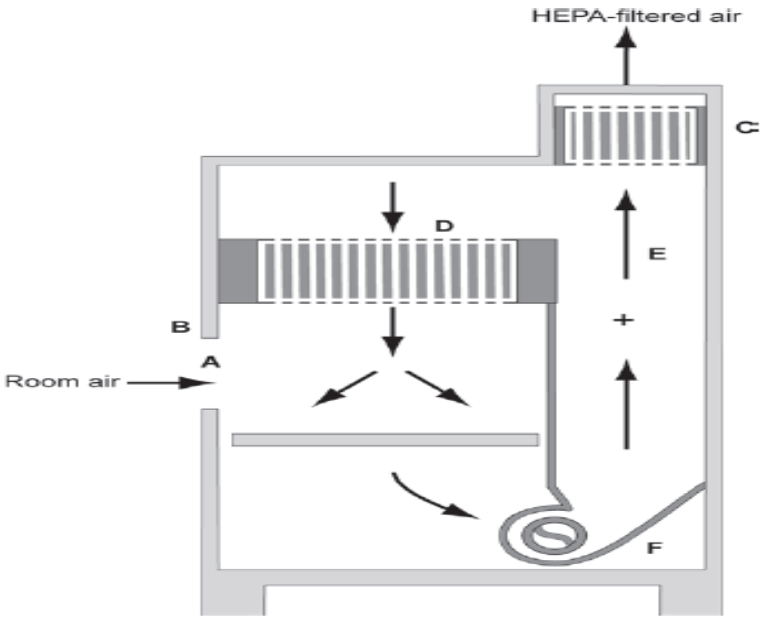


Fig: 2.2 Schematic representation of a Class II A1 biological safety cabinet. A: front opening, B: sash, C: exhaust HEPA filter, D: supply HEPA filter, E: common plenum, F: blower (BMBL 5th edition)

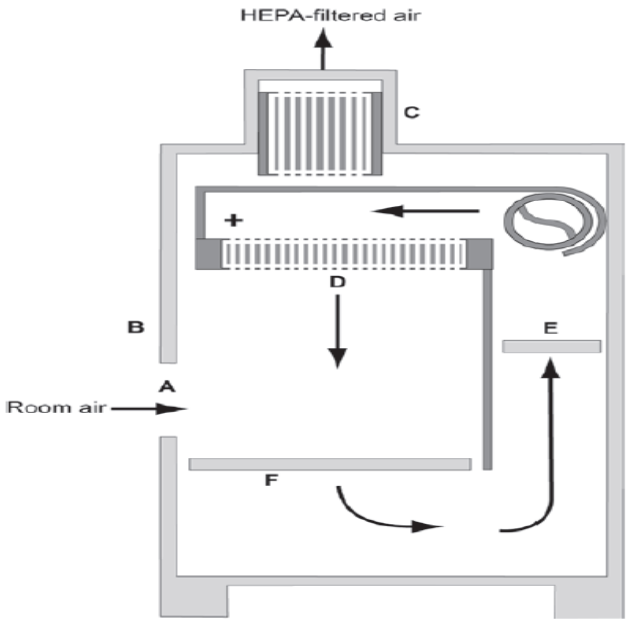


Fig: 2.3 The table top model of a Class II, Type A2 BSC (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) positive pressure common plenum; (F) negative pressure plenum. Note: The A2 BSC should be canopy connected to the exhaust system. (BMBL 5th edition)

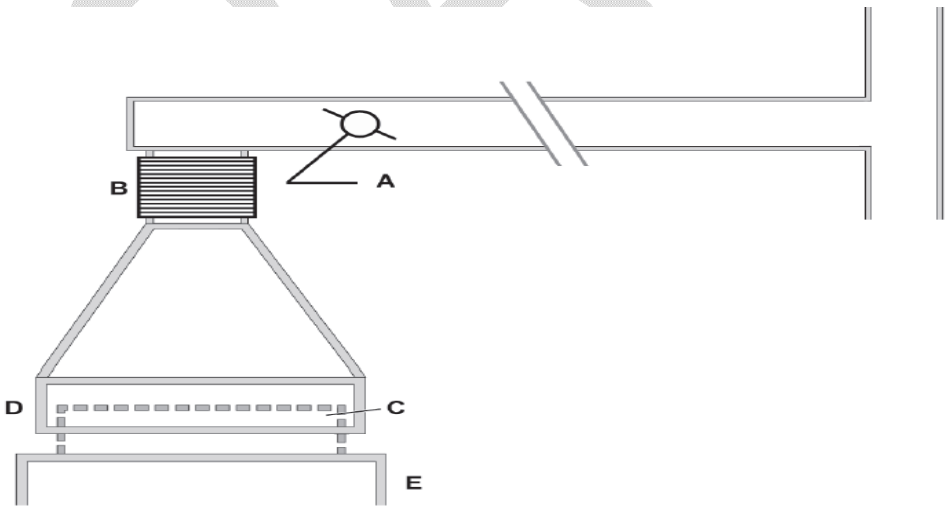


Fig: 2.4 Canopy (thimble) unit for ducting a Class II, Type A BSC (A) balancing damper; (B) flexible connector to exhaust system; (C) cabinet exhaust HEPA filter housing; (D) canopy unit; (E) BSC. *Note:* There is a 1" gap between the canopy unit (D) and the exhaust filter housing (C), through which room air is exhausted. (BMBL 5th edition)

Class II, Type B Cabinets

- Hard-ducted through a dedicated duct exhausted to the atmosphere after passage through a HEPA filter.
- Fans for laboratory exhaust systems should be located at the terminal end of the ductwork to avoid pressuring the exhaust ducts.
- A failure in the building exhaust system may not be apparent to the user, as the supply blowers in the cabinet will continue to operate. A pressure-independent monitor and alarm should be installed to provide warning and shut off the BSC supply fan, should failure in exhaust airflow occur. Since this feature is not supplied by all cabinet manufacturers, it is prudent to install a sensor such as a flow monitor and alarm in the exhaust system as necessary. To maintain critical operations, laboratories using Type B BSCs should connect the exhaust blower to the emergency power supply.

Class II, Type B1 Cabinets (Fig. 1.5)

- Room air is drawn through the face opening of the cabinet at a minimum measured inflow velocity of 100 ft/min.
- Approximately 70 percent of the down flow air exits through the rear grille, passes through the exhaust HEPA filter, and is discharged from the building. The remaining 30 percent of the down flow air is drawn through the front grille for recirculation.

Class II, Type B2 Cabinets (Fig. 1.6)

- This BSC is a total-exhaust cabinet; no air is recirculated within it.
- This cabinet provides simultaneous primary biological and chemical (small quantity) containment. Consideration must be given to the chemicals used in BSCs as some chemicals can destroy the filter medium, housings and/or gaskets causing loss of containment.
- The supply blower draws either room or outside air in at the top of the cabinet, passes it through a HEPA filter and down into the work area of the cabinet.
- All air entering this cabinet is exhausted, and passes through a HEPA filter (and perhaps some other air-cleaning device such as a carbon filter if required for the work being performed) prior to discharge to the outside.
- Hard-ducted through a dedicated duct exhausted to the atmosphere, the exhaust system draws air through both the rear and front grills, capturing the supply air plus the additional amount of room air needed to produce a minimum calculated or measured inflow face velocity of 100 ft/min.
- The higher static air pressure required to operate this cabinet also results in additional costs associated with heavier gauge ductwork and higher capacity exhaust fan.

Therefore, the need for the Class II, Type B2 should be justified by the research to be conducted.

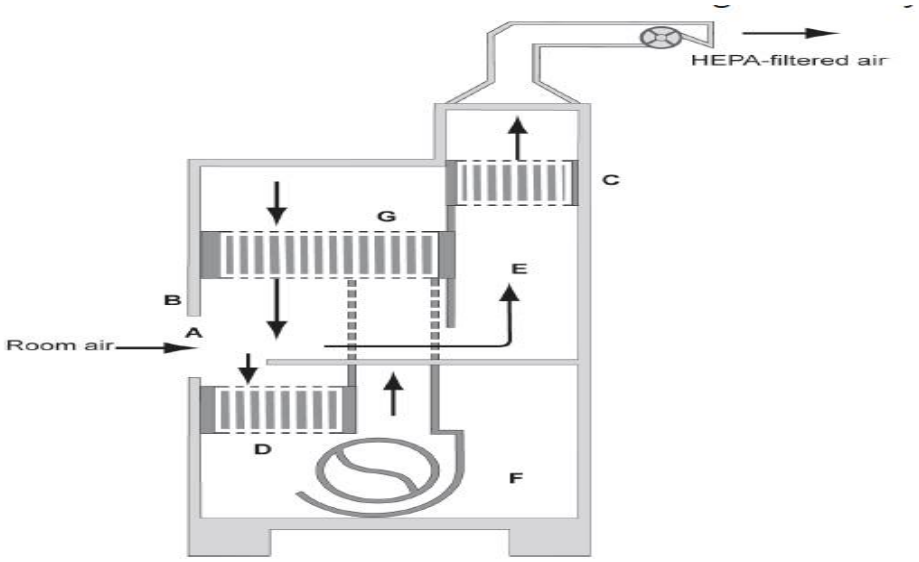


Fig 2.5 The Class II, Type B1 BSC (classic design) (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) negative pressure dedicated exhaust plenum; (F) blower; (G) additional HEPA filter for supply air. Note: The cabinet exhaust needs to be hard connected to the building exhaust system. (BMBL 5th edition)

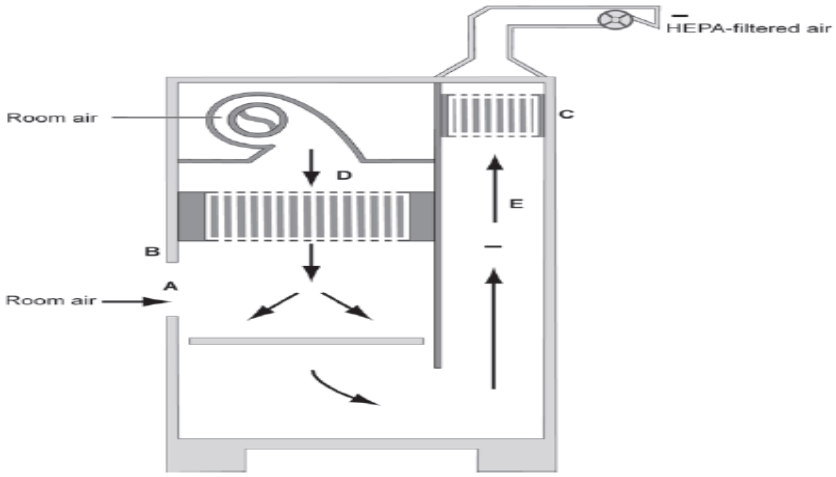


Figure 2.6. The Class II, Type B2 BSC (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) negative pressure exhaust plenum. *Note:* The carbon filter in the exhaust system is not shown. The cabinet needs to be hard connected to the building exhaust system. (BMBL, 5th edition)

Working with Chemicals in BSCs

HEPA filters are effective at trapping particulates and thus infectious agents but do not capture volatile chemicals or gases. Only Type A2-exhausted or Types B1and B2 BSCs exhausting to the outside should be used when working with volatile, toxic chemicals, but amounts must be limited. Also consideration must be given to the chemicals used in BSCs as some chemicals can destroy the filter medium, housings and/or gaskets causing loss of containment.

Minute quantities of volatile toxic chemicals or radionuclides can be used in a Type A2 cabinet only if it exhausts to the outside via a properly functioning canopy connection. In Type B1 cabinet, since the air that flows to the rear grille is discharged into the exhaust system, activities that may generate hazardous chemical vapors or particulates should be conducted toward the rear of the cabinetwork area. Since Type B2 is a total-exhaust cabinet; no air is recirculated within it, thus it provides simultaneous primary biological and chemical (small quantity) containment.

2.1.3 The Class III BSC

- The Class III BSC (Fig. 2.7) was designed for work with highly infectious microbiological agents and for the conduct of hazardous operations and provides maximum protection for the environment and the worker.
- It is a gas-tight enclosure with a non-opening view window. Access for passage of materials into the cabinet is through a dunk tank, that is accessible through the cabinet floor, or double-door pass-through box (e.g., an autoclave) that can be decontaminated between uses.
- Both supply and exhaust air are HEPA filtered on a Class III cabinet. Exhaust air must pass through two HEPA filters, or a HEPA filter and an air incinerator, before discharge directly to the outdoors. Class III cabinets are not exhausted through the general laboratory exhaust system. Airflow is maintained by an exhaust system exterior to the cabinet, which keeps the cabinet under negative pressure (minimum of 0.5 inches of water gauge.)
- Long, heavy-duty rubber gloves are attached in a gas-tight manner to ports in the cabinet to allow direct manipulation of the materials isolated inside. Although these gloves restrict movement, they prevent the user's direct contact with the hazardous materials.

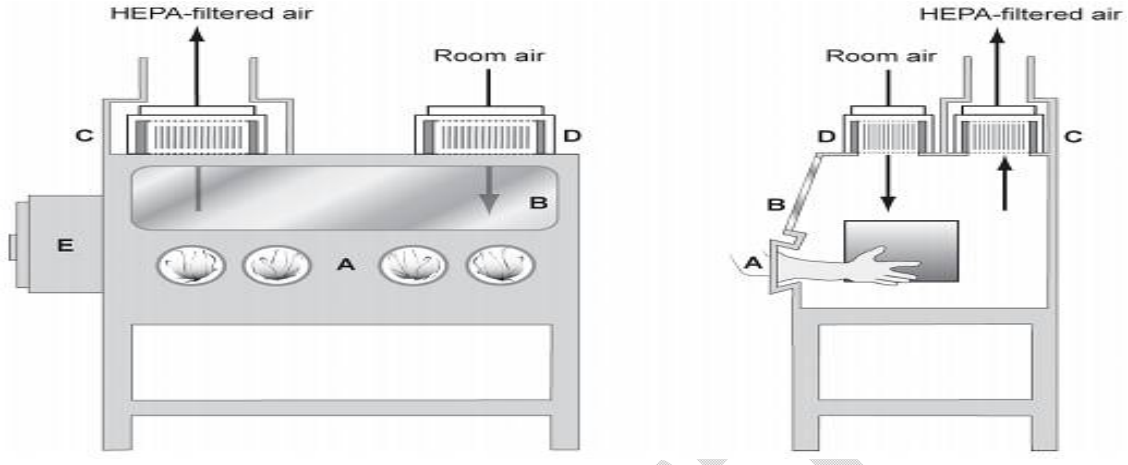


Fig: 2.7 ***The Class III BSC*** (A) glove ports with O-ring for attaching arm-length gloves to cabinet; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) double-ended autoclave or pass-through box. *Note:* A chemical dunk tank may be installed which would be located beneath the work surface of the BSC with access from above. The cabinet exhaust needs to be hard connected to an exhaust system where the fan is generally separate from the exhaust fans of the facility ventilation system. The exhaust air must be double HEPA-filtered or HEPA-filtered and incinerated. (BMBL 5th edition)

2.1.4 Laminar Flow "Clean Bench"

Laminar flow "clean benches" (Fig. 2.8) are not BSCs. These pieces of equipment discharge HEPA-filtered air across the work surface and toward the user. While these units generally have a sash, the air is usually discharged into the room under the sash, resulting in the same potential problems presented by the horizontal laminar flow clean benches. These devices only provide product protection. They can be used for certain clean activities, such as the dust-free assembly of sterile equipment or electronic devices. Clean benches should never be used when handling cell culture materials, drug formulations, potentially infectious materials, or any other potentially hazardous materials. The worker will be exposed to the materials being manipulated on the clean bench potentially resulting in hypersensitivity, toxicity or infection depending on the materials being handled. **Laminar flow "clean benches" must never be used as a substitute for a biological safety cabinet**. Users must be aware of the differences between these two devices.

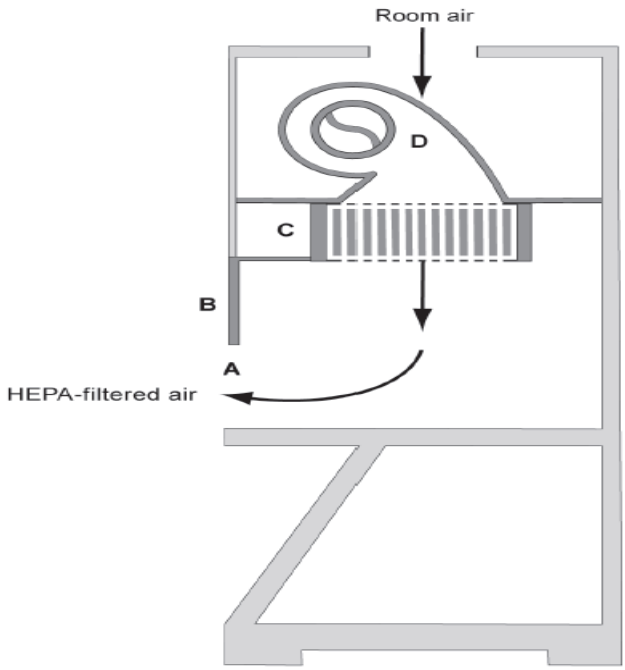


Fig2.8. The vertical laminar flow "clean bench" (A) front opening; (B) sash; (C) supply HEPA filter; (D) blower. *Note:* Some vertical flow clean benches have recirculated air through front and/or rear perforated grilles. (BMBL 5th edition)

2.2 BSC Use: Work practices and Procedures:

2.2.1. Placement of a BSC in the laboratory

- Whenever possible, adequate clearance should be provided behind and on each side of the cabinet to allow easy access for maintenance and to ensure that the cabinet air re-circulated to the laboratory is not hindered.
- When the BSC is hard-ducted or connected by a canopy unit to the ventilation system, adequate space must be provided so that the configuration of the ductwork will not interfere with airflow. The canopy unit must provide adequate access to the exhaust HEPA filter for testing.
- The air curtain created at the front of the cabinet is quite fragile, amounting to a nominal inward and downward velocity of 1 mph. Open windows, air supply registers,

portable fans or laboratory equipment that creates air movement (e.g., centrifuges, vacuum pumps) should not be located near the BSC.

- Other personnel activities in the room (e.g., rapid movements near the face of the cabinet, walking traffic, room fans, open/closing room doors) may also disrupt the cabinet air barrier. Ideally, BSCs should be situated in a location away from common movement of laboratory workers and potentially disturbing air currents.
- The ideal location for the biological safety cabinet is remote from the entry (i.e., the rear of the laboratory away from traffic), since people walking parallel to the face of a BSC can disrupt the air curtain.
- Whenever possible, a 30-cm clearance should be provided behind and on each side of the cabinet to allow easy access for maintenance.
- A clearance of 30–35 cm above the cabinet may be required to provide for accurate air velocity measurement across the exhaust filter and for exhaust filter changes.

2.2.2. Operation of BSC

- If BSCs are not used properly, their protective benefits may be greatly diminished. Operators need to be careful to maintain the integrity of the front opening air inflow when moving their arms into and out of cabinets.
- Cabinets should be turned on at least 5 min before beginning work and after completion of work to allow the cabinet to “purge”, i.e. to allow time for contaminated air to be removed from the cabinet environment.
- The rapid movement of a worker’s arms in a sweeping motion into and out of the cabinet will disrupt the air curtain and compromise the partial containment barrier provided by the BSC. Moving arms in and out slowly, perpendicular to the face opening of the cabinet will reduce this risk.
- The number of movements across the front opening should also be minimized by placing all necessary items into the cabinet before beginning manipulations.
- Before beginning work, the investigator should adjust the stool height so that his/her face is above the front opening.
- Manipulations of materials within BSCs should be delayed for about 1 min after placing hands and arms inside to allow the cabinet to adjust and to “air sweep” the surface of the hands and arms.
- All operations should be performed on the work surface at least four inches in from the front grille. If there is a drain valve under the work surface, it should be closed prior to beginning work in the BSC.

- As a general rule of thumb, keeping clean materials at least one foot away from aerosol-generating activities will minimize the potential for cross-contamination.
- The workflow should be from “clean to dirty”. Materials and supplies should be placed in the cabinet in such a way as to limit the movement of “dirty” items over “clean” ones.

2.2.3. Personal protective equipment while using BSC

- Personal protective clothing should be worn whenever using a BSC.
- Laboratory coats should be worn buttoned over street clothing;
- Latex, vinyl, nitrile or other suitable gloves are worn to provide hand protection. Gloves should be pulled over the wrists of the gown rather than worn inside.
- Masks and safety glasses may be required for some procedures.
- Gloves and gowns must be removed in a manner to prevent contamination of unprotected skin and aerosol generation and wash their hands as the final step in safe microbiological practices.
- Increasing levels of PPE may be warranted as determined by an individual risk assessment. For example, a solid front, back-closing laboratory gown provides better protection of personal clothing than a traditional laboratory coat and is a recommended practice at BSL-3.

2.2.4. Material placement

- Preparing a written checklist of materials necessary for a particular activity and placing necessary materials in the BSC before beginning work serves to minimize the number and extent of air curtain disruptions compromising the fragile air barrier of the cabinet.
- The front intake grill must not be blocked with paper, equipment or other items.
- Materials to be placed inside the cabinet should be surface-decontaminated with 70% Alcohol
- All materials should be placed as far back in the cabinet, towards the rear edge of the work surface, as practical without blocking the rear grill.
- Ideally Aerosol- generating equipment (e.g. mixers, centrifuges, etc.) should not be placed inside the BSC, but if not possible they should be placed towards the rear of 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.
- Active work should flow from clean to contaminated areas across the work surface.
- Materials or equipment placed inside the cabinet may cause disruption of the airflow, resulting in turbulence, possible cross-contamination and/or breach of containment.

Only the materials and equipment required for the immediate work should be placed in the BSC. Extra supplies (e.g., additional gloves, culture plates or flasks, culture media) should be stored outside the cabinet.

2.2.5. Disposable transfer loops

- Open flames must not be used in the BSCs as they create turbulence and disrupt the pattern of HEPA-filtered air being supplied to the work surface and can be dangerous when volatile, flammable substances are also used. For loop sterilization, loop incinerators may be used.
- Disposable sterile loops should be used whenever possible and should be placed in disinfectant after use and discarded as biohazard waste.

2.3. Ultraviolet lights

- UV lamps are neither recommended nor required in Biological Safety Cabinets (BSC). However, if one is using it then it must be cleaned weekly/ regularly to remove the dust.

2.4. Spills

Refer to Chapter 6 on Prevention and management of Laboratory accidents

2.5. Cleaning and disinfection of work surfaces

- All items within BSCs, including equipment, should be surface-decontaminated and removed from the cabinet when work is completed.
- The interior surfaces of BSCs should be decontaminated before and after each use. The work surfaces and interior walls should be wiped with a disinfectant that will kill any microorganisms that might be found inside the cabinet.
- At the end of the work day, the final surface decontamination should include a wipe-down of the work surface, the sides, back and interior of the glass. A solution of 70% alcohol/0.05% sodium hypochlorite or other appropriate disinfectant should be used. A second wiping with sterile water is needed when a corrosive disinfectant, such as bleach/hypochlorite, is used.

2.6. Decontamination of equipment

- BSCs that have been used for work involving infectious materials must be decontaminated before HEPA filters are changed or internal repair work is done. Before a BSC is relocated, a risk assessment considering the agents manipulated

within the BSC must be performed to determine the need and method for decontamination. The most common decontamination method uses formaldehyde gas, although more recently, hydrogen peroxide vapour and chlorine dioxide gas have been used successfully.

2.7. Certification

- Annual certification of BSC is mandatory. In cases where this is not possible, it is better not to work on an open bench, rather than working in a non-certified BSC.
- Some of the existing standards for certification are:
    - National Sanitation Foundation (NSF) Standard #49 for Class II BSCs - was first published in 1976, providing the first independent standard for design, manufacture and testing of BSCs.
    - EN 12469 dated May 2000 - this European standard sets the minimum performance criteria for safety cabinets for work with microorganisms and specifies test procedures for microbiological safety cabinets with respect to protection of the worker and the environment, product protection and cross contamination.
- BSC operation needs to be verified at the time of installation and, as a minimum, annually thereafter.
- The operational integrity of a BSC must be validated before it is placed into service and after it has been repaired or relocated. Relocation may break the HEPA filter seals or otherwise damage the filters or the cabinet.
- Each BSC should be tested and certified at least annually to ensure continued, proper operation.
- On-site field-testing must be performed by experienced, qualified personnel.
- Education and training programs for persons seeking accreditation as qualified to perform all field certification tests are offered by a variety of organizations. Selecting competent individuals to perform testing and certification is important.
- The annual tests applicable to each of the three classes of BSCs are listed in Table 2.1.

Table 2.1. Field Performance Tests Applied to the 3 classes of BSCs (Biosafety in Microbiological and Biomedical Laboratories, 5th Ed, CDC, US)

Test performed for	BSC I	BSC II	BSC III
1. Primary containment			
• Cabinet integrity	n/a	a (A1 Only)	a
• HEPA filter leak	Required	Required	Required
• Down Flow velocity	n/a	Required	n/a
• Face Velocity	Required	Required	n/a
• Negative Pressure/ventilation rate	b	n/a	Required
• Airflow Smoke Patterns	Required	Required	e, f
• Alarms and interlocks	c, d	c, d	Required
Electrical Safety			
• Electrical leakage	e, d	e, d	e, d
• Ground fault interrupter	d	d	D
Other			
• Lighting Intensity	e	e	E
• UV Intensity	c, e	c, e	c, e
• Noise level	e	e	E
• Vibration	e	e	e

Required Required during certification

a Required for proper certification if the cabinet is new, has been removed for maintenance

b If used with gloves

c If present

d Encouraged for electrical safety

e Optional, at the discretion of the user

f Used to determine air distribution within cabinet or clean to dirty procedures

n/a Not applicable

Chapter 3

Good Microbiological Techniques-Safe laboratory procedures

Human error, poor laboratory techniques and misuse of equipment and not following laboratory safety instructions cause the majority of laboratory injuries and work-related infections. This chapter provides the information on the technical methods that are designed to avoid or minimize the most commonly reported problems of this nature.

3.1 Laboratory safety

Safety in the laboratory requires every employee's participation and cooperation. Non-compliance with safety precautions not only endangers the individual, but also compromises the health and safety of fellow workers. All staff of the laboratory shall follow the Good Microbiological Techniques (GMTs) which include the following:

3.1.1 Entry / access to laboratory area

- The international biohazard warning symbol (Fig 3.1) and sign must be displayed on the doors of the rooms where high risk microorganisms are handled.
- Entry to laboratory working area should be restricted only for laboratory persons.
- Doors of the laboratory should be kept closed.
- Children should not be allowed to enter laboratory working areas.

Fig 3.1 International biohazard warning symbol

3.1.2 Laboratory Design and Facilities

- Enough space should be available.

- Laboratory should have smooth easily cleanable walls, ceiling and floors impermeable to liquids and resistant to chemicals and disinfectants. Floors should be slip resistant.
- Bench tops should be impervious to water and resistant to disinfectants, acids, alkalies and organic solvents.
- Ample illumination should be available for laboratory procedures.
- Storage space must be adequate to hold supplies for immediate use and hence prevent overcrowding on bench tops. Additional long term storage space at a convenient location should also be available.
- Regular water supply should be available.
- Wash basins with running water, should be provided in the laboratory room preferably near the exit door
- Suitably equipped first aid box should be available in the laboratory.
- Rodents and insects control procedure in the laboratory should be in place.
- In Biosafety level 2 facility, an autoclave should be available in appropriate proximity to the laboratory.
- Fire and electricity emergency safety, emergency shower and eyewash facility should be suitably addressed.
- Emergency electricity supply should be ensured with suitable power backup.

3.2 Standard Precautions

Standard Precautions represent the minimum infection prevention measures that apply to all patient care, regardless of suspected or confirmed infection status of the patient, in any setting where healthcare is delivered. Standard Precautions replaces earlier guidance relating to Universal Precautions and Body Substance Isolation.

Standard Precautions include: 1) use of personal protective equipment (e.g., gloves, gowns, facemasks), depending on the anticipated exposure, 2) hand hygiene, 3) safe techniques/practices.

3.2.1 Personal Protective Equipment (PPE)

Personal Protective Equipment (PPE) may act as barrier to minimize the risk of exposure. The clothing and equipment selected is dependent on the nature of work performed, type of the pathogen and its transmissibility. PPE should be worn when working in the laboratory. It should be removed and hands should be washed before leaving the laboratory.

(A.) Gloves

Can reduce the incidents of contamination of hands but cannot prevent penetrating injuries by needles and other sharp instruments.

Gloves should be:

- Well-fitting disposable gloves.
- Heavy duty general purpose rubber gloves for washing infected glassware/sharps

Uses of Gloves

- Worn while collecting/handling blood specimens, blood soiled items or whenever there is a possibility of exposure to blood or body fluids containing blood. (Fig 3.2)
- Worn while disposing laboratory waste

When to change gloves

- Must be changed if visibly contaminated with blood/breached
- The heavy duty gloves may be decontaminated and reused but should be discarded if they are peeling, cracked, discolored, or if they have puncture, tears etc.
- Should be removed before handling door knobs, telephones, pens, performing office work and leaving the laboratory.

(B). Laboratory gowns

- Laboratory gowns prevent contamination of clothing.
- Laboratory gowns or uniforms (preferably wrap-around gowns) should be worn when in the laboratory and should be removed before leaving.
- Front opening lab coats/gowns must be buttoned up while working in the laboratory and must be with full sleeves
- Plastic aprons should be used while cleaning infected re-usable items and during disposing wastes.

(C) Facial protection

- Facial protection reduces the impact and splash on face/eyes/mouth
- Simple protective glasses/goggles or face shields may be worn if splashing or spraying of blood/body fluids is expected. These should not be worn outside the laboratory.

(D) Masks

Masks if used correctly may protect the user from aerosol/droplet borne/air-borne infection. The particular type of mask to be used is related to particular risk profile of the category of personnel and his/her work. The risk categorization may change according to the expected degree of environmental contamination and lethality of the agent.

Two types of masks are recommended for various categories of personnel depending upon the work environment;

- Triple layer surgical mask
- N 95 Respirator

N-95 and triple layer mask is used while handling of patient's specimens who are suspected of novel influenza viruses.

(E).Occlusive bandage

All skin defects e.g. Cuts, scratches or other breaks must be covered with water-proof dressing before handling infectious materials.

3.2.2. Hand washing

Hand washing is the single most important means of preventing the spread of infection. Hands should be washed between patient contacts and after contact with blood/ body fluids, secretions, excretions and equipment or articles contaminated by these. (Annexure 1)

- The role of hands in the transmission of infections has been well demonstrated, and can be minimized with appropriate hand hygiene.
- Hands should be washed thoroughly in running water with soap without missing any area.
- Washing of hands is mandatory after
    - Contamination with blood / body fluids
    - After removing gowns / coats and gloves
    - Before eating / drinking and leaving the laboratory

The following facilities are required for handwashing:

- Running water: large washbasins preferably with hands free controls, which require little maintenance and with anti-splash devices.
- Products: dry soap or liquid antiseptic depending on the procedure. - Ideally, liquid soap dispensers should be provided in the laboratories, which should be regularly cleaned and maintained. If not feasible, soap bars after washing should

be left in a dry tray to prevent contamination with microorganisms which grow in moist conditions

- Suitable material for drying of hands; disposable towels, reusable sterile single use towels or roller towels which are suitably maintained.

3.3 Safe techniques:

- All procedures and manipulations of potentially infectious material should be performed in a biosafety cabinet / well ventilated area, to minimize the formation of droplets, aerosols, splashes or spills.
- Mouth pipetting should be strictly prohibited. Mechanical pipetting devices should be used for pipetting of all liquids in the laboratory.
- Centrifugation should be done in tubes with safety caps.

3.3.1. Handling of sharps

- Extreme care should be used to avoid auto-inoculation.
- All chipped or cracked glassware should be discarded in appropriate containers.
- Broken glass should be picked up with a brush and pan. Hands must never be used.
- The disposable needles should never be recapped or removed from the syringes. Before discarding disposable syringes/needles, have to be shredded, cut or mutilated. This ensures that they are not recycled / reused.
- Sharps have to be discarded in a puncture proof container with an effective chemical disinfectant (1% sodium hypochlorite).
- Used needles should be discarded in puncture-proof rigid containers (plastic or cardboard boxes) after disinfection in 1% freshly prepared sodium hypochlorite solution (common bleach). Do not mix with other waste.
- The used sharps should never be passed directly from one person to another. A kidney tray may be used for this purpose.
- Each Health Care Worker should dispose of his/her own sharps.
- Sharp disposable containers should be located close to the point of use.
- Sharp disposal containers should be sent for disposal when three-fourth full.

3.3.2. Specimen collection and handling

Improper collection, transport and handling of specimens in the laboratory not only carry a risk of infection to the personnel involved but also will not be useful for testing/diagnosing infectious organism.

3.3.2.1 Specimen collection

- Specimen, specially blood and body fluids should be collected in pre-sterilized screw-capped **plastic containers** properly sealed to prevent spillage or leakage.
- Specimen containers should be robust and should not leak when the cap or stopper is correctly applied. **Alway**s grasp the tube or outside of the specimen container, not the stopper or cap, when picking up tubes or specimen containers to prevent spills and breakage.
- Ensure tops are tightly secured on all specimen containers, blood-collection tubes, and specimen tubes before advancing for analysis or storage.
- Request a new specimen if a specimen container is broken or has spilled its contents.
- Document the incident, and notify the supervisor if an exposure occurred.
- In case of a highly precious specimen if the container shows evidence of breakage, leakage or soiling, it should be transferred with a gloved hand into a second sterile container. Any important information should be rewritten from the old to the new container.
- Containers should be correctly labelled to facilitate identification. (Fig 3.2)
- Do not keep the specimens on requisition forms. Figure 3.3
- If the requisition slip is contaminated with blood, it should be rejected. In case of emergency, the contaminated slip may be handled using gloves.
- Hands should be thoroughly washed with soap and water before and after handling specimens.
- If the outside of the container is visibly **contaminated** with blood it should be cleaned with disinfectant. All blood specimens should be placed in small leak-proof impervious plastic tubes for transportation to the laboratory. Preferably blood specimens should be collected in vacutainer tubes.
- Specimen request or specification forms should not be wrapped around the containers but placed in separate, preferably waterproof envelopes/ zip locks whenever the specimen needs to be transported.

Patient' name	Identification No.
Specimen type	
Date of collection	Time

Fig 3.2. Labelling and identification of specimens

Fig 3.3: Do not keep specimens on request forms

3.3.2.2 Specimen transport within the facility

- To avoid accidental leakage or spillage, secondary leak proof containers, should be used so that the specimen containers remain upright.
- The secondary containers may be of metal or plastic, should be autoclavable or resistant to the action of chemical disinfectants, and the seal should preferably have a gasket. They should be regularly decontaminated.
- The outer container should be rigid and sturdy.

3.3.2.3 Receipt of specimens in the laboratory

- Laboratories that receive large numbers of specimens should have a designated room or area for this purpose. It is preferable to have computerized system for record maintenance.

- Leaking specimen containers, requisition forms smeared with specimens, and improperly labeled specimen containers should not be accepted.

3.3.2.4 Opening specimen packages

- Personnel who receive and unpack specimens should be aware of the potential health hazards involved, and should be trained to adopt standard precautions, particularly when dealing with broken or leaking containers.
- Primary specimen containers should be opened preferably in a biological safety cabinet if not available must be opened while wearing proper PPE.

3.3.2.5. Separation of serum

- Always follow standard operative procedure and take care of following
    - Hands, eye and mucous membrane protection should be worn.(Standard precaution)
    - Splashes and aerosols can only be avoided or minimized by good laboratory techniques.
    - Blood and serum should be pipetted carefully, not poured. Pipetting by mouth must be forbidden.
- Pipette tips/disposable pipetters must be disposed in a discarding jar containing suitable disinfectant like 1% sodium hypochlorite.

3.3.2.6. Films and smears for microscopy

Fixing and staining of blood, sputum and faecal specimens for microscopy do not necessarily kill all organisms or viruses on the smears. These items should be handled with forceps, stored appropriately, and decontaminated and/or autoclaved before disposal.

3.3.3 Handling of Lyophilized biological material

Opening vials of freeze-dried (lyophilized) material can be hazardous because these fine dry powders are easily dispersed into the atmosphere when air rushes into the evacuated vessel. The following procedure may be used to safely open a vial containing lyophilized material.

- Place the ampule/vial and the suggested diluent (water or medium as appropriate) in a BSC
- Wear gloves, face mask and laboratory coat when opening lyophilized vials.
- Remove the aluminum crimp from the vial. Discard the crimping material into the sharps container.

- Cover the stopper with a moistened gauze pad, and carefully lift the edge of the stopper and allow air to slowly enter the vial. Do not disturb the contents of the vial.
- Once the vacuum has been released, remove the stopper completely and place the stopper upside down on absorbent paper/towel dipped in disinfectant.
- Add the appropriate amount of diluent to the vial using a sterile pipette.
- Replace the stopper and allow the vial contents to hydrate for several minutes.
- Discard the gauze and absorbent paper with other contaminated materials.
- Using a pipette, transfer the contents of the vial to an appropriate container.
- Discard the original vial with other contaminated materials.

3.4. General Biosafety instructions for laboratory workers

- Eating, drinking, smoking and application of cosmetics are prohibited in the laboratory.
- Sandals and open style shoes do not afford proper foot protection and are not to be used.
- As far as possible lenses should not be worn in eyes instead one should wear spectacles.
- Laboratory and work tables should be scrupulously cleaned with liquid detergents and disinfectants. Laboratory work surface should be decontaminated once a day after completion of day's activity and immediately after spill of viable material with disinfectant.
- Paper work should not be done on potentially contaminated surface.
- Clean / dirty areas must be clearly demarcated
- All work surfaces in daily use such as bench tops, sinks, and trolleys etc. must be disinfected with a disinfectant designed for laboratory use at the end of each work shift. (0.5%) dilution of sodium hypochlorite or bleach is used as disinfectant if specimens of blood, blood products or body fluids have been handled.
- Cuts in hands should be properly covered with waterproof adhesive bandage

Chapter 4

Sterilization and Disinfection Procedures

4.1 Definitions

Cleaning is a process which removes foreign material (e.g. soil, organic material, micro-organisms) from an object.

Disinfection: Chemical means of killing microorganisms, but not necessarily spores.

Disinfectant: A chemical or mixture of chemicals used to kill microorganisms, but not necessarily spores. Disinfectants are usually applied to inanimate surfaces or objects. Disinfectants used should be in proper concentration and for suitable period of time.

High level disinfection: High-level disinfection traditionally is defined as complete elimination of all microorganisms in or on an instrument, except for small numbers of bacterial spores.

Sterilization: A process that kills and/or removes all classes of microorganisms and spores.

4.2 Environmental cleaning

Cleaning is the removal of dirt, organic matter and stains. Cleaning includes brushing, vacuuming, dry dusting, washing or damp mopping with water containing a soap or detergent. Dirt, soil and organic matter can shield microorganisms and can interfere with the killing action of decontaminants (antiseptics, chemical germicides and disinfectants). Floors, surfaces, sinks and drains should be cleaned with water and detergent. Routine use of disinfectants is unnecessary.

4.3 Hand washing

Good hand washing is the most important step in preventing disease transmission in health settings. Hands must be washed after handling biohazardous materials, and before leaving the laboratory.

In most situations, thorough washing of hands with ordinary soap and water is sufficient to decontaminate them, but the use of germicidal soaps is recommended in high-risk situations. Hands should be thoroughly lathered with soap, using friction, for at least 40 seconds, rinsed in clean water and dried using a clean paper or cloth towel (if available, warm-air hand-dryers may be used).

Alcoholic hand rubs are not a substitute for hand washing, except for rapid hand decontamination between patient contacts.

4.4 Decontamination of disposable items

4.4.1 Used needles and syringes

- Do not detach needle from syringe
- Do not recap the needle
- Cut the used needle in a needle cutter
- Dispose of the cut needle into a puncture proof, tamper proof sharp container for autoclaving followed by shredding
- Dispose the syringe in red bag for autoclaving followed by shredding

4.4.2 Other disposable items

Materials required

- 1% sodium hypochlorite
- Glass jar
- Bio-waste bag (puncture resistant with appropriate color code)
- Gloves

Procedure

- Freshly prepare requisite quantity of disinfectant in a jar meant for this purpose
- Label the jar with name of disinfectant and date of preparation
- Put articles to be discarded in the solution for minimal contact time/overnight
- Drain off disinfectant
- The material for disposal to be put in appropriate coloured biohazard bags & dispose off as per Biomedical waste management rules

Note

Always prepare fresh solution of disinfectant before use as ready to use solution has shorter shelf life, compared to concentrated one and will be of no use if not freshly prepared. Care should be taken while handling & preparing the solution as it may be corrosive to skin.

4.5 Decontamination of reusable articles contaminated with infectious material

4.5.1 Lab coats

Material required

- Disinfectant as described above
- Metallic box/ Tray
- Bunsen burner (Heating device for boiling)

Procedure

After treating the material with suitable solution of disinfectant for 1-2 hours/overnight, proceed further as follows

- Drain off disinfectant in sink fitted with tap.
- Alternatively immerse in boiling water for 15-20 min

- Allow cooling the material in metallic box/ tray
- Drain off water
- Pass on the material for washing
- Materials can also be autoclaved before washing.

4.5.2 .Glassware containing culture material

Materials required

- Puncture proof and autoclavable containers

Procedure

- Discard all the glassware contaminated with culture material directly into puncture resistant and autoclavable containers/metal box. Place box/container with material to be decontaminated in autoclave designated for this work only. Decontaminate the material by autoclaving. Drain off leftover liquid appropriately and pass on material for further washing.

Note

Be sure that decontamination should only be done with autoclaves designated for this purpose. Autoclave should be checked for its efficacy using commercially available chemical indicator strips with every autoclave cycle (show colour change indicating appropriate autoclaving) and biological indicator at least once every week.

4.6. Common Disinfectants and their use

Table 4.1 Common Disinfectants and their use

Disinfectant	Articles	Comments
Sodium hypochlorite	• Disinfection of glassware contaminated with blood and body fluids	• Should be used in well ventilated areas • Protective clothing must be worn while handling undiluted • Not to be mixed with strong acids to avoid release of chlorine gas • Corrosive to metals
Bleaching powder (7gm/litre of water with 70% available chlorine, may be used in place of liquid bleach if liquid bleach is not available)	Toilets, bathrooms	Same as above
Alcohol (70%) Isopropyl alcohol, ethyl alcohol, methylated spirit	• Smooth metal surfaces, table tops and table tops on which sodium hypochlorite cannot be used	• Flammable, toxic, to be used in well ventilated areas, avoid inhalation • To be kept away from heat sources, electrical equipment, flames, hot surfaces • Should be allowed to dry completely
Chlorhexidine Combined with alcohol or detergents	Disinfection of skin and hands	• Most commercially available preparations contain large amounts of alcohol (70%) and are flammable. • Do not use them or store them near a flame, heater, or electrical device. • Apply in a well-ventilated place.

Formula for dilution of Stock solution of Sodium Hypochlorite to working concentration of Sodium Hypochlorite

Amount of stock required = Working Conc. Required * Working solution Volume required/ Stock Conc.

Water Required = Working solution Volume Required − Amount Stock Required

Table 4.2 Preparation of required dilution of Na Hypochlorite from stock solutions.

Required Strength (Available solution of chlorine	**Stock/commercially available Sodium Hypochlorite**		
	4 %(40g/L); dilute	**5 %(50g/L); dilute**	**6%(60g/L); dilute**
0.1%(1 g/L)	1:39*	1:49	1:59
0.5%(5 g/L)	1:7	1:9	1:11
1%(10 g/L)	1:3	1:4	1:5

**parts of stock solution: parts of water*

4.7. Washing of laboratory glassware

The type of glassware i.e. new and dirty/ used is subjected to washing for further use. The method used for each type is described below.

4.7.1. New glassware

Purpose

Usually new glassware is slightly alkaline in nature. Before washing, this alkaline nature has to be neutralized for final use.

Material required

- 2% hydrochloric acid
- Big plastic basin
- Demineralized water
- Hot air oven for drying purpose only

Procedure

- Prepare sufficient quantity of 2 % hydrochloric acid (e.g. 98 ml of water & 2.0 ml hydrochloric acid) as per the requirement in a big plastic basin

- Wash the newly received glassware under running tap water to remove the visible dust sticking inside and/or outside surface of the article
- Soak the already washed articles in 2% hydrochloric acid solution
- Leave them there overnight
- Take the articles from 2 % hydrochloric acid and rinse in clean water twice
- Finally wash using demineralized water. Allow to dry using hot air oven
- Pass on for packing & sterilization for further use

Note

- Care should be taken while using HCl
- Add acid to water drop by drop by constant stirring (and not vice versa)

4.7.2. Dirty glassware

Material required

- 1 % detergent solution.
- Cotton or aluminum foil for plugging
- Washing brush
- Good quality water supply
- Hot air oven
- Draining rack
- Wire basket for drying
- Demineralized water.

Procedure

- Take material, glassware etc. already decontaminated (chemically/ autoclaving) and rinse twice in lukewarm water to remove any dirty stain sticking on them
- Put the material to be washed in bowl containing 1% detergent solution
- Allow to boil
- While in solution, scrub inside & outside surface of the glassware with the help of the brush
- Leave the glassware in the solution for 2 - 3 hrs
- Take out each article one by one and rinse under running tap water till no trace of detergent is left, which otherwise may lead to false results when used.
- Drain the water by putting each article on a draining rack or by keeping articles upside down in a wire basket
- Put articles in wire basket and keep in hot air oven at 60°C for drying purpose only

- Take out each article and plug using non-absorbent cotton/aluminum foil
- Sterilization of glassware can be done using dry heat or by autoclaving.

4.8. Methods of Sterilization

Sterilization is carried out by steam under pressure, dry heat, gas or liquid chemicals. The choice of the methods like autoclaving, use of hot air oven etc. depends on a number of factors including type of material of the object, number and types of organisms involved and risk of infection to patients or staff. Any sterilization procedure should be monitored routinely by mechanical, chemical and biological techniques. Sterilized items should be protected against recontamination.

Depending upon the nature of material to be sterilized, sterilization procedures used in microbiology laboratory can be divided into the following categories.

- Dry heat
- Moist heat
- Filtration

4.8.1. Dry heat

The commonly used methods to sterilize the material are as follows

- Flaming
- Hot air sterilization.

4.8.1.1. Flaming

Purpose

Used to sterilize material, such as, inoculating wire/ loop, tip of the forceps, searing iron, scalpel etc.

Material required

Bunsen burner attached to gas supplies.

Match box.

Procedure

Light the burner with the help of match box. Adjust the cone of the flame to blue. Hold the inoculum wire/ loop/ tip of the forceps etc. vertically and heat till it gets red hot. Allow to cool before use. Put off the flame

Note

Each time when heating in the Bunsen burner flame, allow to cool down the instrument. Check loop/ wire etc. by touching a portion of the medium to be inoculated. Heat the loop vertically

so that the entire length of the loop is heated. Dip the loop in disinfectant solution before heating to avoid splattering

4.8.1.2 Hot air Sterilization

Purpose

The method is used for sterilizing the material like dry glass test tubes, Petri dishes, flasks, glass pipettes, all glass syringes etc. and instruments like forceps, scalpels etc.

Procedure

Arrange the material (pre washed & packed) to be sterilized, loosely and evenly on the racks of the oven so that air can circulate properly and heat the load evenly in the oven. Note the time when desired temperature is reached (Heating time). Hold the load on the same temperature for the specified period as mentioned below.

Temperature Holding Time

160 °C for 60 minutes.

170 °C for 40 minutes.

180 °C for 30 minutes.

The most common temperature for hot air oven for sterilization is 160 °C for 60 min. On completion of the holding time period, switch off the power supply and allow the oven to cool down slowly. Open the Hot air oven not before the temperature has come down to 80 °C and take out the sterilised material. Put down the date of sterilization on each packet and store in dust free area for future use. Maintain daily records of the equipment/ material sterilized as per the Performa given below:

Date	Details of items to be sterilised	Temperature at which sterilization was done	Starting time	Time when the set temperature is reached	Time when switched off	Holding time	Chemical indicator tape (colour changed)

Precautions:

- Dry up all the material before putting into sterilization in hot air oven
- Don't place heat sensitive material inside the oven

- As air is poor conductor of heat, do not pack the material to be sterilized in the oven too tightly
- After holding time is over, hot air oven is switched off, wait until the temperature of the oven falls below 80°C. Only then open the door of the oven to take out the material otherwise opening immediately after holding time leads to breaking of the glassware and may also cause injuries to the operator

4.8.2 Moist heat

Moist heat or steam under pressure is one of the most efficient methods of sterilization. Depending upon the material to be sterilized moist heat can be applied in different forms as discussed below.

Autoclave

Autoclaves use pressurized steam to destroy microorganisms, and are the most dependable systems available for the decontamination of laboratory waste and the sterilization of laboratory glassware, media, and reagents.

Principle

When water boils, its vapor pressure is equal to surrounding atmospheric pressure. When boiling is done in a closed vessel, there is increase in the inside pressure of vessel which raises the temperature of boiling water above 100°C.

Saturated steam under pressure is more efficient way of sterilization as compared to dry heat because it provides greater lethal action. It is quicker in heating up the exposed articles. It penetrates the porous material such as cotton wool, stoppers, paper, cloth wrapper etc.

Type of Autoclaves

The two basic types of steam sterilizers (autoclaves) are the gravity displacement autoclave and the high-speed prevacuum sterilizer. In the former, steam is admitted at the top or the sides of the sterilizing chamber and, because the steam is lighter than air, forces air out the bottom of the chamber through the drain vent. The gravity displacement autoclaves are primarily used to process laboratory media, water, pharmaceutical products, regulated medical waste, and nonporous articles whose surfaces have direct steam contact. For gravity displacement sterilizers the penetration time into porous items is prolonged because of incomplete air elimination. For example to decontaminate 4 Kg of microbiological waste requires at least 45 minutes at 121°C because the entrapped air remaining in a load of waste greatly retards steam permeation and heating efficiency. The high-speed prevacuum sterilizers are similar to the gravity displacement sterilizers except they are fitted with a vacuum pump (or ejector) to ensure air removal from the sterilizing chamber and load before the steam is

admitted. The advantage of using a vacuum pump is that there is nearly instantaneous steam penetration.

The ability of the sterilizer to reach physical parameters necessary to achieve sterilization should be monitored by mechanical, chemical, and biological indicators.

Temperature Pressure Time

Recognized minimum exposure periods for sterilization are 30 minutes at 121°C in a gravity displacement sterilizer or 4 minutes at 132°C in a prevacuum sterilizer.

Note

- At the end of holding time switch off the power supply
- Allow the autoclave to cool slowly which can be seen by gradual decrease in pressure till it shows zero reading
- Allow the wrapping paper to be dried
- Put date on each article and place in dust free area for future use
- Ensure that air from chamber has been expelled completely because air steam mixture has a lower temperature than steam e.g. temperature of 50% air & 50% steam mixture will be 112°C instead of 121°C provided by the pure steam
- As the simple autoclave lack means for drying the load after sterilization, it is therefore important to avoid placing sterilized articles in contact with unsterilized objects/ surface unless the wrapping is dried
- To check the efficacy of autoclave, each cycle should be run using chemical indicator tape

4.8.3 Quality control

In order for a product to be considered sterilized, it is necessary to verify that all the stages of the sterilization process have been carried out correctly. To verify that these have been fulfilled, various tests have been developed to evaluate the characteristics of the process. The steam cycle is monitored by mechanical, chemical, and biological monitors. Steam sterilizers usually are monitored using a printout (or graphically) by measuring temperature, time and pressure. To monitor effectiveness of the cycle, chemical indicator tapes are used, change of colour uniformly indicates that the process has been completed satisfactorily.

4.8.3.1. Chemical indicators

Most commonly, chemical indicators for steam sterilization/hot air oven are printed inks on packaging materials, or paper strips on which the chemical indicator is printed. A feature of paper strip indicators is that they can be placed inside packs being sterilized and thus checked

by the end user. Chemical indicators need to be used for every item in every sterilized load combined with an electronic printout of sterilization parameters for each load. One has to keep in mind that chemical indicators by themselves do not guarantee that the sterilization process complied with all the requirements: personnel who use these must receive precise training to allow them to determine if the result obtained is coherent with the evolution of the whole sterilization process.

4.8.3.2. Biological indicators

Biological monitoring is the use of living microorganisms for checking and challenging a sterilization process. The goal in using biological indicators is to determine whether all of the microorganisms have been killed during the sterilization process. These are considered the best methods for controlling the quality of a sterilization process. The microorganism based biological indicator is a system in which a large number of living hard-to-kill spores of a chosen bacterial species are presented either in a small paper envelope or in a self-contained vial. *Geobacillus stearothermophilus* (formerly *Bacillus stearothermophilus*), a hardy spore, is the organism of choice when monitoring steam sterilization. Bacterial spores of *B. atrophaeus (*also called *Bacillus subtilis var niger)* is used with dry heat. The spore indicator is placed in the sterilizing load. The deactivation of spores during the sterilization stage is indicated by their inability to grow in a suitable growth medium over a long incubation time (8 to 72 hours) following the sterilization cycle. These tests are standardized and manufacturers indicate how to use them and interpret the results.

Frequency of use of quality control indicators in the sterilization processes:

Mechanical: Temperature, Time, Pressure in each sterilization cycle

Chemical: In each package.

Biological: Weekly.

Autoclave Log:

Date	List of items to be sterilized	Starting Time	Time at which desired temp is reached	Holding time completed at	Indicator tape	Sign

Indicator Tape

Chapter 5

Biomedical waste management

Laboratory waste is a potential reservoir of pathogenic microorganisms and requires appropriate handling. The commonest documented transmission of infection from waste to health care workers is through contaminated needles.

Infectious waste can transmit numerous diseases in the community and also to those who handle waste. Besides, the increasing use of disposables in health care is also posing an additional burden on the waste management facility. It is extremely important that the unscrupulous reuse of these disposable items is prevented.

5.1 National Rules/Regulatory mechanism for biomedical waste management in India:

- The Government of India, in the erstwhile Ministry of Environment and Forests, in exercise of the powers conferred by section 6, 8 and 25 of the Environment (Protection) Act, 1986 (29 of 1986), published the Bio-Medical Waste (Management and Handling) Rules via notification in July 1998. These provided a regulatory framework for management of bio-medical waste generated in the country.
- On 28th March 2016, the aforesaid Ministry published in the Gazette of India, the Bio-Medical Waste Management Rules, 2016. These rules are in supersession of the Bio-Medical Waste (Management and Handling) Rules, 1998, except as respects things done or omitted to be done before such suppression.
- These rules apply to all persons who generate, collect, receive, store, transport, treat, dispose, or handle bio medical waste in any form including hospitals, nursing homes, clinics, dispensaries, veterinary institutions, animal houses, pathological laboratories, blood banks, Ayush hospitals, clinical establishments, research or educational institutions, health camps, medical or surgical camps, vaccination camps, blood donation camps, first aid rooms of schools, forensic laboratories and research laboratories.
- Safe and proper identification, handling, storage, and disposal of biomedical waste from laboratories and related facilities is the responsibility of every occupier. "Occupier" means a person having administrative control over the institution and the premises generating bio-medical waste, which includes a hospital, nursing home, clinic, dispensary, veterinary institution, animal house, pathological laboratory, blood bank, health care facility and clinical establishment, irrespective of their system of medicine and by whatever name they are called. Duties of the Occupier are detailed in Annexure 2.
- As per the Bio-Medical Waste Management Rules, 2016, the biomedical waste includes the categories mentioned in Schedule 1 of the notification (Annexure 3) namely:

- **Yellow category**: Human anatomical waste, Animal anatomical waste, Soiled waste, Expired or discarded medicines, Chemical waste, Chemical liquid waste, Discarded contaminated beddings and Microbiology, biotechnology and other clinical waste,
- **Red category:** includes contaminated recyclable waste,
- **White category:** includes waste sharps including metals,
- **Blue category:** includes glassware and metallic body implants.

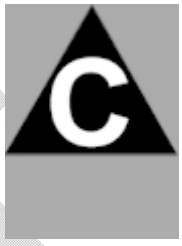
5.2. Management of biomedical waste

5.2.1. Waste segregation at point of generation

- Hospital/ laboratory waste requires management at every step from generation, segregation, collection, transportation, storage, and treatment to final disposal.
- Of the waste generated in health care settings, approximately 25% is infectious but if not segregated properly, the entire waste becomes infectious thereby escalating the overall cost of waste management. The most practical approach to the management of biomedical waste is to identify and segregate infectious waste**,** which would in turn drastically reduce the cost of the waste disposal in health care settings.
- Biomedical waste should be segregated into containers or bags at the point of generation in accordance with Annexure 3. This includes placing different types of waste in different containers or colour-coded-bags at the site of generation.
- Proper segregation should identify waste according to source and type of disposal/ disinfection. (Annexure 3).
- Colour coded bags as per national norms (Annexure 3) need to be placed in appropriate containers with the appropriate label/logo e.g. biohazard symbol for infectious waste (Fig. 5.1).
- Puncture proof containers made of plastic or metal with a biohazard symbol, in blood collection areas, injection trolleys, nursing stations and operation theatres should be made available for collecting metallic wastes.
- Syringes should be either mutilated or needles should be cut and or stored in tamper proof, leak proof and puncture proof containers for sharps storage.
- Ensure segregation of liquid chemical waste at source and ensure pre-treatment or neutralization prior to mixing with other effluent generated from health care facilities.

The bio-medical waste containers or bags must be labeled as below:

Fig 5.1. LABEL FOR BIO-MEDICAL WASTE CONTAINERS or BAGS

BIOHAZARD SYMBOL	CYTOTOXIC HAZARD SYMBOL
	
BIOHAZARD	**CYTOTOXIC**

HANDLE WITH CARE

Note : Label shall be non-washable and prominently visible.

5.2.2 Collection bags

- Color coded bags used for collection of segregated waste must be free of chlorine. Solid waste is collected in leak-resistant heavy duty bags. Coloured bags made of non-chlorinated plastic with biohazard sign and labels mentioning date and details of waste are to be used. The bags are tied tightly after they are three- fourth full.

5.2.3 Pre-treatment, Packing, storage and transport

Microbiology waste, clinical laboratory waste including, blood specimens, blood bags and other highly infectious waste must be pre-treated by sterilization for 6 log reduction or through disinfection for 4 log reduction of microbial load, as per the World Health Organisation (WHO) guidelines before packing and sending to the common bio-medical waste treatment facility. Standards for autoclaving are as given in Schedule II of Biomedical Waste Management Rules 2016 (Annexure 5).

Provision must be made within the premises of health care facility for a safe, ventilated and secured location for storage of segregated biomedical waste in colored bags or containers, inaccessible to scavengers and protected against insects, birds, animals and rain, to ensure that there is no secondary handling, pilferage of recyclables, or inadvertent scattering or spillage by animals. The bio-medical waste from such place or premises should be directly transported to the authorized common bio-medical waste treatment facility for the appropriate treatment and disposal.

Transport of biomedical waste to common bio-medical waste treatment facility will be done only in vehicles having appropriate label as provided in Schedule IV of Bio-Medical Waste Management Rules 2016, along with necessary information as specified in part B of the Schedule IV of Bio-Medical Waste Management Rules 2016 (Annexure 3). The vehicles used for transportation must comply with conditions if any stipulated by the State Pollution Control Board or Pollution Control Committee in addition to the requirement contained in the Motor Vehicles Act, 1988, if any or the rules made there under for transportation of such infectious waste.

Untreated human anatomical waste, animal waste, soiled waste and biotechnology waste shall not be stored beyond a period of 48 hrs.

5.2.4. Treatment and disposal

- On site biomedical waste treatment and disposal facilities are not to be established unless a common bio-medical waste treatment facility is not available within a distance of 75kms.
- The duties of the common biomedical waste treatment facilities are at Annexure 4.
- The laboratory and highly infectious bio-medical waste generated shall be pre-treated by equipment like autoclave or microwave before handing over segregated waste (as per the Annexure 3) to common bio-medical waste treatment facility for treatment, processing and final disposal:
- The health care facilities and common bio-medical waste treatment facility must treat and dispose of the bio-medical waste in accordance with Schedule I (Annexure 3), and in compliance with the standards provided in Schedule-II of the Bio-Medical Waste Management rules 2016 (Annexure 5).
- Every operator of common bio-medical waste treatment facility shall set up requisite biomedical waste treatment equipment like incinerator, autoclave or microwave, shredder and effluent treatment plant as a part of treatment, prior to commencement of its operation. The standards for treatment and disposal of bio-medical wastes in Schedule III of Bio-Medical Waste Management Rules 2016 must be complied with.
- The handling and disposal of all the mercury waste and lead waste is to be done in accordance with the respective rules and regulations.

5.3 Bio-medical waste handlers

- Immunise all health care workers and others, involved in handling of biomedical waste for protection against diseases including Hepatitis B and Tetanus that are likely to be transmitted by handling of bio-medical waste, in manner as prescribed in the National

Immunization Policy or the guidelines of the Ministry of Health and Family Welfare issued from time to time.

- Ensure occupational safety of all health care workers and others involved in handling of bio-medical waste by providing appropriate and adequate personal protective equipment.
- Conduct health check up at the time of induction and at least once in a year for all health care workers and others involved in handling of bio-medical waste and maintain the records for the same.

5.4 Annual Report

- **E**very occupier or operator of common bio-medical waste treatment facility has to submit an annual report to the prescribed authority in Form-IV (refer to Bio-Medical Waste Management Rules 2016), on or before the 30th June of every year. The prescribed authority is state Pollution Control Boards in respect to states and Pollution control Committees in respect to Union Territories. For establishments under Ministry of Defence, the prescribing authority is Director General, Armed Forces Medical Services.
- The Annual Reports must also be uploaded on the websites of Occupiers.

5.5 Maintenance of records

- Maintain and update on day to day basis the bio-medical waste management register and display the monthly record on the website, the biomedical waste generated in terms of category and color coding.
- Records related to the generation, collection, reception, storage, transportation, treatment, disposal or any other form of handling of bio-medical waste, must be maintained for a period of five years.
- All records must be available for inspection and verification by the prescribed authority or the Ministry of Environment, Forest and Climate Change at any time.
- Maintain all records for operation of incineration, autoclaving etc. for a period of 5 years.

5.6 Reporting of accidents:

- In case of any major accident at any institution or facility or any other site while handling bio-medical waste, it must be intimated immediately to the prescribed authority and forward a report within twenty-four hours in writing regarding the remedial steps taken in Form I of Bio-Medical Waste Management Rules 2016.
- Information regarding all other accidents and remedial steps taken shall be provided in the annual report.

5.7 Training:

- All workers involved in handling of biomedical waste must be provided training at the time of induction and at least once a year thereafter.
- Records of the training programmes conducted, number of personnel trained and number of personnel not undergone any training must be maintained.

Chapter 6
Prevention & Management of Laboratory accidents

The risk of laboratory accidents in the workplace is real, always present, and an integral part of working in a diagnostic laboratory, and in particular the clinical microbiology laboratory. The potential cause can be primarily biological, chemical or electrical. Diagnostic laboratories can be safe places to work if standard and appropriate safe work practices and procedures are easily accessible, understood by employees, enforced, and followed. These procedures are to be properly outlined in laboratory SOPs/manuals. These plans are composed of essential elements related to preventing an exposure, and, equally important, they describe employer and employee involvement and responsibilities before and after an exposure. Timely and appropriate actions taken can greatly reduce or even eliminate the chance of laboratory accidents. Important aspects of laboratory safety which can prevent accidents and how to manage them starts from receipt of specimens and are described below.

6.1 Slip, Trip and Fall Hazards

Slips, trips, and falls can cause a laboratory worker to drop or spill vessels containing infectious agents or dangerous chemicals. They can also lead to skin punctures and abrasions that make laboratory workers more vulnerable to LAIs. Common causes of laboratory slips/trips/fall hazards include:

- Mats if they are not properly anchored to the floor.
- Wet floor
- Walking on paper, cardboard, or packaging materials
- Obstructed view, poor lighting, clutter in the walkway, mats or other items in the walkway, uncovered cables, open drawers or cabinets, and uneven walking surfaces

To prevent these hazards:

- Promptly remove water from floor
- Keep drawers and cabinets closed except when they are being accessed.
- Store material appropriately in the laboratory
- Do not use alcohols to clean floors; alcohols will dissolve floor wax, creating areas with different degrees of traction.

6.2 Electrical Safety

- Electrical hazards can be categorized into two main types: those that can result in an electrical shock and those that can cause fires and/or explosions.

- Electrical shocks can be avoided by ensuring that all equipment and electrical installations are inspected and tested regularly including earthing/grounding systems.
- Do not overload electrical circuits. Minimize or eliminate the use of multi-outlet power strips. When power strips are necessary, the safety office of the facility or a licensed electrician must approve their use.
- Because electrical devices can generate sparks, do not use them near flammable or volatile gases or liquids.
- Never place flammable liquids in a household refrigerator. The spark generated by the door-activated light switch can ignite fumes trapped in the unit, causing an explosion and fire.
- Fire extinguisher for electrical fires must be installed

Note:

Upon learning of the threat of fire within the building, laboratory personnel will, to the extent possible:

- Turn of all gas burners, biological safety cabinets, electric motors, and other electrical equipment.
- Place containers of infectious materials into autoclaves, incubators, refrigerators, freezers or other storage areas.
- Leave the laboratory as quickly as possible using designated fire evacuation routes.
- Use fire extinguisher and inform the appropriate authority.

6.3 Biological Hazards

6.3.1. Sharp injuries

6.3.1.1. Common causes

Skin punctures and cuts can directly introduce an infectious agent into the body and can provide a route whereby a secondary agent can enter. This could be due to:

- **Needle stick injuries**
    - Needle stick injuries occur most often during recapping of needles after use.
    - Do not recap needles. If recapping is absolutely required, single hand technique may be used by placing the cap on the table and recap the needle to minimize injury and accidental inoculation.
- **Handling broken containers**
    - When handling broken containers with spilled infectious substances, adhere to the following guidelines:

- Wear appropriate gloves for this procedure (based on risk assessment and protection needed).
- Cover the broken container and spilled infectious substance with a cloth or with paper towels.
- The cloth or paper towels and the broken material should be cleared away into biohazard sharps container. Fragments of glass are to be handled with forceps, not gloved hands.

- **Handling Pasteur pipettes**
  - Both the top and the bottom of a Pasteur pipette can cause puncture wounds.
  - Before handling a glass Pasteur pipette, examine the top of the pipette to see if it is broken or cracked. Broken pipettes can produce puncture wounds.
  - Dispose of used Pasteur pipettes in leak- and puncture-resistant containers. In most locations, contaminated Pasteur pipettes are considered sharps and must be disposed of as such.
  - Whenever possible, substitute with plastic pipetters.

- **Handling other sharp devices**
  - Knives, scissors, and tissue homogenizers are frequently used to dissect tissue specimens before testing. These items must be handled carefully in order to prevent cuts and skin punctures that could injure or inoculate laboratory workers with infectious materials.
  - Pointed forceps are often used for fine dissection and for removing coverslips. These forceps can puncture the unwary user, causing injury and/or infection.
  - Glass slides can break and puncture skin and so must be handled carefully.

6.3.1.2. Emergency management

Puncture wounds, cuts and abrasions

- The affected individual should remove protective clothing, wash the hands and any affected area(s), apply an appropriate skin disinfectant, and seek medical attention as necessary.
- The cause of the wound and the organisms involved should be reported, and appropriate and complete medical records kept.

Needle stick injury: following steps should be taken

- Needle stick injury and cuts should be washed with soap and water
- Splashes to the nose, mouth or skin should be flushed with water.
- Eyes should be irrigated with clean water, saline or sterile irrigants
- Pricked finger should not be squeezed or put into mouth reflexly.

6.3.1.3. Reporting of the Exposure

All percutaneous or mucocutaneous exposures should be reported using the form at Annexure 7

- **In case of needle stick**
  - Find out the status of **HIV, HBV and HCV** of the source patient and the health care worker. Ensure confidentiality of laboratory reports so that the HCW is not discriminated.
  - In case of HIV negative patient, baseline HIV test of HCW should be done on day of exposure, 6^{th} week and 12^{th} week. Simultaneously health worker should receive basic regime of prophylaxis.
  - In case of HIV positive patient the exposure to the appropriate authority should be informed and condition must be treated as an emergency. Prompt reporting is essential because in some cases, HIV **post exposure prophylaxis (PEP**) may be recommended and it should be started as soon as possible preferably within a few hours. The decision to start PEP is made on the basis of degree of exposure and the HIV status of the source from whom the exposure / infection has occurred. Drugs for PEP must be available with emergency medical officer who should be available round the Clock.
  - Similarly if patient is HBV positive the appropriate authority should be informed. In a non-immune health care worker (not previously immunized) immunoglobulin for HBV must be administered at the earliest (preferably within 7-8 hrs) followed by full course of hepatitis B vaccination.
  - For HCV post exposure management, the HCV status of the source and the exposed person should be determined, and for HCW exposed to an HCV positive source, follow-up HCV testing should be performed to determine if Infection develops. Immunoglobulin and antiviral agents (e.g., interferon with or without ribavirin) are not recommended for PEP of hepatitis C.

For more details regarding management of needle stick injuries refer to:
http://www.naco.gov.in/NACO/National_AIDS_Control_Program/PEP_full/

6.3.2. Spill Response Procedures for Infectious agents

6.3.2.1. Spill in a Biosafety Cabinet (Note: ***Leave the cabinet turned on.)***

1. Don double gloves, a lab coat, and eye protection if not already wearing them.

2. Cover spilled material with an absorbent paper towel. Once the absorbent material is in place, wet material with 10% solution of sodium hypochlorite or other appropriate disinfectant. Let stand for 15-20 minutes, wipe up and wash surface with appropriate disinfectant.
3. If personnel are contaminated, remove potentially contaminated garments at the BSC and decontaminate garments or place in autoclave bag for autoclaving. Wash hands and other potentially exposed skin surfaces thoroughly with soap and water. Don fresh PPE, return to worksite, and spray walls, liners, and equipment with an appropriate disinfectant.
4. If necessary, flood the work surface, drain pan and catch basin below the work surface with disinfectant. Allow at least 15-20 minutes contact time.
5. Soak up the disinfectant and drain the catch basin into a container. Lift the front exhaust grille and tray and wipe all surfaces. Ensure that no foreign materials are blown into the area below the grille.
6. If a 10% bleach solution is used on metal surfaces, rinse with water or 70% ethanol after decontamination is complete.
7. If the spill overflows into the interior of the cabinet, more extensive decontamination of the cabinet may be necessary.

6.3.2.2. Spill in the Laboratory

1. In case of spill in the laboratory, ***leave the room immediately, lock the door, post a warning sign and inform your supervisor.*** If clothing is contaminated, remove and turn the exposed side of fabric in on itself and place in autoclave bag or biohazard container. Wait at least 30 minutes before reentering the laboratory to allow dissipation of aerosol created by the spill.
2. Don fresh gloves, a lab coat, and eye protection.
3. Carefully lay towels over the spill and pour the disinfectant liberally on the towel and around the spill. Use more concentrated disinfectant if the volume of material will significantly dilute the disinfectant.
4. Allow 15-20 minutes contact time.
5. Use forceps to place sharp objects into a sharps container. Using a dustpan and dust broom, tongs, etc., transfer all contaminated materials (paper towels, gloves, labware, etc.) biohazard waste containers for removal.
6. Wipe surrounding surfaces with disinfectant to cover all splash areas. Wipe flat surfaces to remove any material that may have splashed out and settled on those surfaces.

7. Place all contaminated materials, including protective clothing, into an autoclave bag/disinfectant or disposed off in biohazard waste container.
8. Wash hands with soap and water.

6.4 Gases in the Laboratory:

6.4.1. Compressed Gas Cylinders

Compressed CO_2 cylinders are often used to provide gases for CO_2 incubators; the risks associated with these incubators are minimal as long as the room is well ventilated.

- Gas cylinders pose three major safety hazards:
  - Gas cylinders are heavy; thus, a falling cylinder can cause injury.
  - The valve attached to the cylinder is relatively fragile compared with the cylinder; if the valve is broken off, the cylinder can become a dangerous projectile.
  - Faulty valves or regulators can leak, allowing toxic or flammable gases to enter the room.

Minimizing hazards

Many of these potential hazards can be minimized by adoption of safe handling practices.

- Cylinders must be securely anchored to the wall with chains or straps to prevent falling. Cylinders <18 inches tall may be secured in stands or wall brackets.
- Regulators of gas cylinders are normally supplied with instructions for routine maintenance and periodic checking to ensure safe operation. Follow these instructions and checks carefully.
- Always use specially designed cylinder carts when moving cylinders. Cylinders must be secured to the cart and the valve covers must be in place when moving them. They are not to be dragged, rolled, or physically carried. Do not lift cylinders by the cap.

6.4.2. Liquid Gases (Cryogens)

Cryogenic liquids are liquefied gases that have a normal boiling point below -238°F (-150°C). Liquid nitrogen is used in the microbiology laboratory to freeze and preserve cells and virus stocks. The principal hazards associated with handling cryogenic fluids include cold contact burns and freezing, asphyxiation, explosion, and material embrittlement.

Cold contact burns and freezing

- Liquid nitrogen is dangerously cold (-320°F [-196°C]), and skin contact with either the liquid or gas phase can immediately cause frostbite

- Always wear eye protection (face shield or safety goggles). The eyes are extremely sensitive to freezing, and liquid nitrogen or liquid nitrogen vapors can cause eye damage.
- Do not allow any unprotected skin to contact uninsulated piping, hoses, tongs, specimen box storage racks, or other metal objects because these become extremely cold when exposed to liquid nitrogen. Skin will stick to the metal, tearing the flesh when one attempts to withdraw from it.
- When filling cryogenic dewars (specialised types of vacuum flask used for storing cryogens (such as liquid nitrogen or liquid helium), whose boiling points are much lower than room temperature) , wear long-sleeved shirts or laboratory coats, long trousers (preferably without cuffs which could trap the liquid), closed shoes (never sandals or open shoes), and insulated cryogloves labeled as appropriate for use with cryogenic liquids. Do not tuck pant legs into shoes or boots; doing so could direct liquid into the foot coverings and trap the cryogenic liquid against the skin.
- Wear loose-fitting thermal gloves with elbow-length cuffs when filling dewars. Ensure that gloves are loose enough to be thrown off quickly if they contact the liquid.
- Never place gloved hands into liquid nitrogen or into the liquid nitrogen stream when filling dewars. Gloves are not rated for this type of exposure. Insulated gloves are designed to provide short-term protection during handling of hoses or dispensers and during incidental contact with the liquid. Use special cryogenic liquid tongs when retrieving items from liquid nitrogen.
- Liquid nitrogen confers a high risk of splattering; jets of liquid nitrogen can be generated when cans, canisters, and other objects that are at much higher temperatures are placed into liquid nitrogen. These activities can present a freezing hazard.
- Do not insert a hollow tube into the liquid nitrogen because liquefied gas may spurt from the tube.

6.5 Asphyxiation hazards

- Although nitrogen is nontoxic and inert, it can act as an asphyxiant by displacing the oxygen in the air to levels below that required to support life. Inhalation of nitrogen in excessive amounts can cause dizziness, nausea, vomiting, loss of consciousness, and death without warning.
- When liquid cryogens are expelled into the atmosphere at room temperature, they evaporate and expand to 700–800 times their liquid volume. Even small amounts of liquid can displace large amounts of oxygen gas and decrease the oxygen content of the atmosphere below a safe level

- Do not store dewars or nitrogen containers in a confined space. The venting gas could displace enough oxygen to become a hazard.
- If enclosed spaces must be used, install oxygen monitors. Train personnel to leave the area immediately if the alarm sounds. The alarm must be audible both inside and outside the room to prevent anyone from entering the room.

6.6 Explosion hazards

- Liquid gases, even those considered inert, can present explosion hazards.
- Do not drop, tip, or roll containers on their sides; doing so could damage the vessel and/or cause a sharp increase in internal pressure.
- Cryotubes and glass ampoules used for freezing cells and viruses can explode without warning when removed from cryogenic storage. These tube explosions are presumed to be caused by entry of liquid nitrogen into the tube through minute cracks; as the tube thaws, the rapidly expanding gas causes the tube to explode, scattering the contents of the tube. Whenever possible, store ampoules in the gaseous phase rather than submerging in the liquid nitrogen of the cryogenic dewar. An imperfectly sealed ampoule will pick up less nitrogen in the gaseous phase.
- Place cryotubes and ampoules onto gauze or paper toweling in an autoclavable, heavy-walled container immediately after removal from the nitrogen tank, and close the lid of the heavy-walled container quickly. If an explosion occurs, autoclave the entire vessel.

6.7. Instrumentation

Whether automated or manual, procedures with the potential for producing specimen aerosols and droplets (e.g., stopper removal, vortexing, opening or piercing evacuated tubes, using automatic specimen dispensers) require use of appropriate PPE and engineering controls designed to prevent exposure to infectious agents.

6.7.1 Water baths and water humidification pans in CO_2 incubators

- Clean regularly even if disinfectants are added to the water.
- To reduce bioburden, add disinfectant such as a phenolic detergent to the water as needed. Avoid using sodium azide to prevent growth of microorganisms because it forms explosive compounds with certain metals.
- Raise the temperature to 90°C or higher for 30 minutes once a week for decontamination purposes.
- Water baths and humidification pans in CO_2 incubators can harbor bacteria, algae, and fungi that become aerosolized when the water bath lid or incubator doors are opened. These aerosols can contaminate cultures and the environment.

- Empty and clean water baths and humidification pans regularly to minimize organism buildup and the production of biofilms that are notoriously difficult to remove.

6.7.2 Centrifuges and cytocentrifuges

- Centrifuges can be extremely dangerous instruments if not properly cleaned, maintained and operated. Laboratory staff must be trained in centrifuge operation and the hazards associated with centrifugation.
- Operators are to have documented training and competency assessments on each type of centrifuge they operate. Documented instruction for each centrifuge type includes proper instrument startup and shutdown, emergency procedures and shutdown, balancing of tubes, use of safety cups and covers, rotor and container selection, requirements for high-speed and ultracentrifuges, and container fill-height limitations.
- Operate all high-speed and ultracentrifuges on a stable, resonance-free surface (floor, bench top, or heavy table) with at least 6-inch clearance at the sides and 4 inches at the rear of the centrifuge.
- In BSL-2 or higher containment laboratories, rotors need aerosol containment (like use of "O-rings" and gasketed safety cups).
- Load and unload rotors in a BSC, particularly in virology and mycobacteriology sections.
- Clean centrifuges at the end of each shift and immediately after a spill.
- Never operate centrifuges with visible spills of blood or body fluid present.
- Maintain a complete and comprehensive rotor log for every high-speed and ultracentrifuge rotor to include all user names, run dates, durations, speeds, total rotor revolutions, and any notes on rotor condition.
- Tube breakage during centrifugation presents the greatest risk for contamination because large aerosol clouds are produced. Occult contamination can occur when centrifuging tubes without gasket safety caps.
- Provide a centrifuge spill kit containing a disinfectant compatible with the centrifuge materials, heavy duty gloves, tweezers or forceps, cotton, broom, hand brush, and dustpan.
- If a specimen tube breaks within the plastic screw-capped canister or bucket in a centrifuge, take the following steps.
    - Turn the motor off and allow time for aerosols to settle before opening the centrifuge.
    - Remove the canister and place in a BSC if available otherwise place the canister in a well ventilated area using appropriate PPE.

- Notify a supervisor or senior person in charge and other colleagues working in the area.
- While wearing protective clothing, open the canister.
- Pour a 1% hypochlorite or a noncorrosive disinfectant into the canister to decontaminate all surfaces; let the canister soak in bleach or disinfectant solution for 20 minutes. Clean the canister thoroughly.
- Do not pick up broken glass with gloved hands. Use forceps or cotton held in forceps, or tongs and dispose off into a appropriate sharps container.
- Discard all nonsharp contaminated materials from canister into a yellow biohazard bag for biohazard waste disposal.
- Swab or wipe unbroken capped tubes with the same disinfectant; then swab or wipe again, wash with water, and dry.
- All materials used during the cleanup must be treated as infectious waste.

6.8. Essential components of a Contingency plan for managing laboratory accidents

The contingency plan should provide operational procedures for:

1. Precautions against natural disasters, e.g. fire, flood, earthquake
2. Biohazard risk assessment
3. Incident-exposure management and decontamination
4. Emergency evacuation of people from the premises
5. Emergency medical treatment of exposed and injured persons
6. Medical surveillance of exposed persons
7. Clinical management of exposed persons
8. Epidemiological investigation
9. Post-incident continuation of operations.

In Nutshell:

- Never assume a laboratory injury or exposure is insignificant or unimportant.
- Employees must be empowered to report all incidents, with the goal of protecting themselves, their colleagues, and their families without fear of reprisal. Report all exposures to the supervisor immediately, and discuss the exposure to determine what, if any, actions need to be taken. Actively participate in the documentation of the exposure, and provide pertinent information that will be used in the development of the corrective-action plan
- Cooperate fully with the laboratory's approved post exposure processes, and follow prudent medical advice

Chapter 7
Transport of Infectious substances

Transport of infectious and potentially infectious substances is subject to regulations and these need to be followed when Laboratory personnel ship infectious substances.

Compliance with the rules will:

1. Reduce the likelihood that packages will be damaged and leak
2. Reduce the exposures resulting in possible infections
3. Improve the efficiency of package delivery

This chapter provides information for classifying infectious substances for transportation and ensuring their safe packaging. They stress the importance of developing a working relationship between those involved – the sender, the carrier and the receiver – in order to provide for safe and expeditious transport of these materials.

7.1 Classification of Infectious Substances

Infectious substances are divided into the following categories:

7.1.1. Category A (Infectious substances)

An infectious substance which is transported in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals. (Annexure 7)

The proper shipping name should be INFECTIOUS SUBSTANCE, AFFECTING HUMAN or INFECTIOUS SUBSTANCE, AFFECTING ANIMALS only

7.1.2. Category B (Biological substances)

An infectious substance which does not meet the criteria for inclusion in Category A. Examples include human or animal excreta or

The proper shipping name should be “BIOLOGICAL SUBSTANCE, CATEGORY B”.

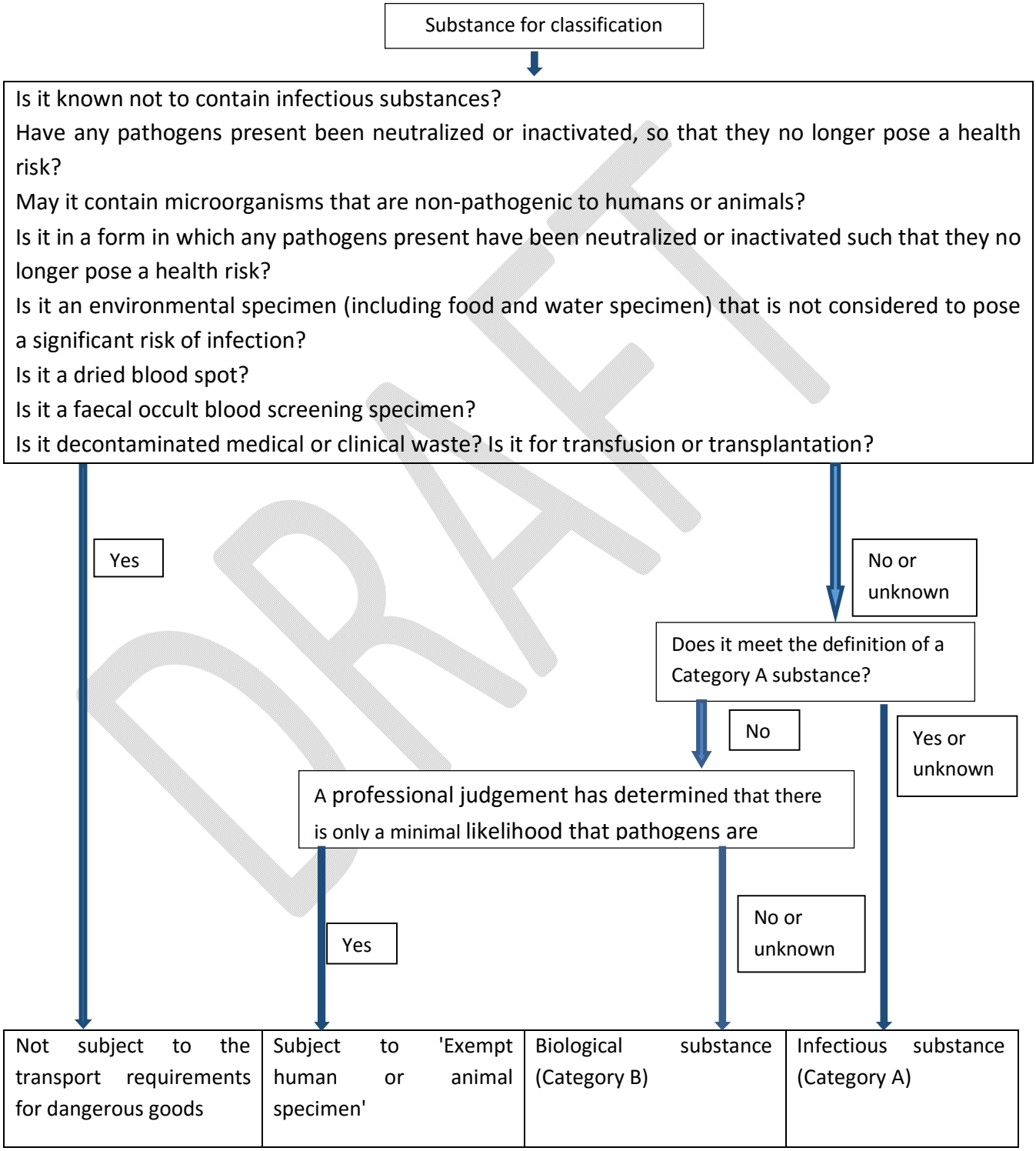
7.1.3. Exemptions

1. Substances that do not contain infectious substances or that are unlikely to cause disease in humans or animals

2. Substances containing microorganisms which are non-pathogenic to humans or animals
3. Substances in a form, where any present pathogens have been neutralized or inactivated such that they no longer pose a health risk
4. Environmental specimens (including food and water specimens) which are not considered to pose a significant risk of infection
5. Dried blood spots
6. Faecal occult blood screening specimens
7. Blood or blood components which have been collected for the purposes of transfusion or for the preparation of blood products to be used for transfusion or transplantation and any tissues or organs intended for use in transplantation
8. Decontaminated medical or clinical waste

There are NO packaging requirements for these full exemptions unless a professional judgement has determined that there is only a minimal likelihood that pathogens are present; in which case triple package.

7.2. Flowchart for the classification of infectious substances and patient specimens (WHO Guidelines for the safe transport of Infectious substances and diagnostic specimens, 2015)



7.3. General preparation of shipments for transport

Because of the differences in the hazards posed by Category A and Category B infectious substances, there are variations in the packaging, labelling and documentation requirements for the two categories. The packaging material used for **Category A** substances require more stringent specifications like Pressure tested at 95 kPa, Drop tested from 9 metres, Puncture tested at 7 kg and Stacking tested.

7.3.1 Basic triple packaging system

This system of packaging shall be used for all infectious substances. It consists of three layers as follows:

- Primary container: A primary watertight, leak-proof container containing the specimen. After tightening the cap, apply sealing tape to seal the cap. The container is packaged with enough absorbent material (e.g. cotton wool) to absorb all fluid in case of breakage or leakage.
- Secondary packaging. A second durable, watertight, leak-proof packaging to enclose and protect the primary container(s). Place inside secondary plastic containers with screw-capped lids/Ziploc bags the sealed specimens cushioned with absorbent material. Specimens from different patients should never be sealed in the same bag. In case specimens from several patients have to be packed inside the same secondary plastic container, the specimens have to be double-bagged properly in sealed plastic bags, Place additional absorbent material inside the secondary container to cushion multiple primary receptacles and absorb any leakage that may occur. Tape the laboratory request form sealed in a plastic bag to the outside of this secondary container.
- Outer packaging. Secondary packaging are placed in outer shipping packaging with suitable cushioning material. The smallest overall external dimension shall be 10 x 10 cm. It should have a resistant, high-density external cover (e.g. metal, wood, or fireboard), shock-absorbent padding on the inside, and a tight- fitting lid. The outer package must be leak-proof and well insulated, and can contain ice, cold packs or dry ice when required. EPI vaccine carriers or other commercially made containers may be used as a tertiary container to transport. **Vaccine carriers that have been used for specimen transport must never be reused for carrying vaccines.**

Each completed package is normally required to be correctly marked, labelled and accompanied with appropriate shipping documents (as applicable).

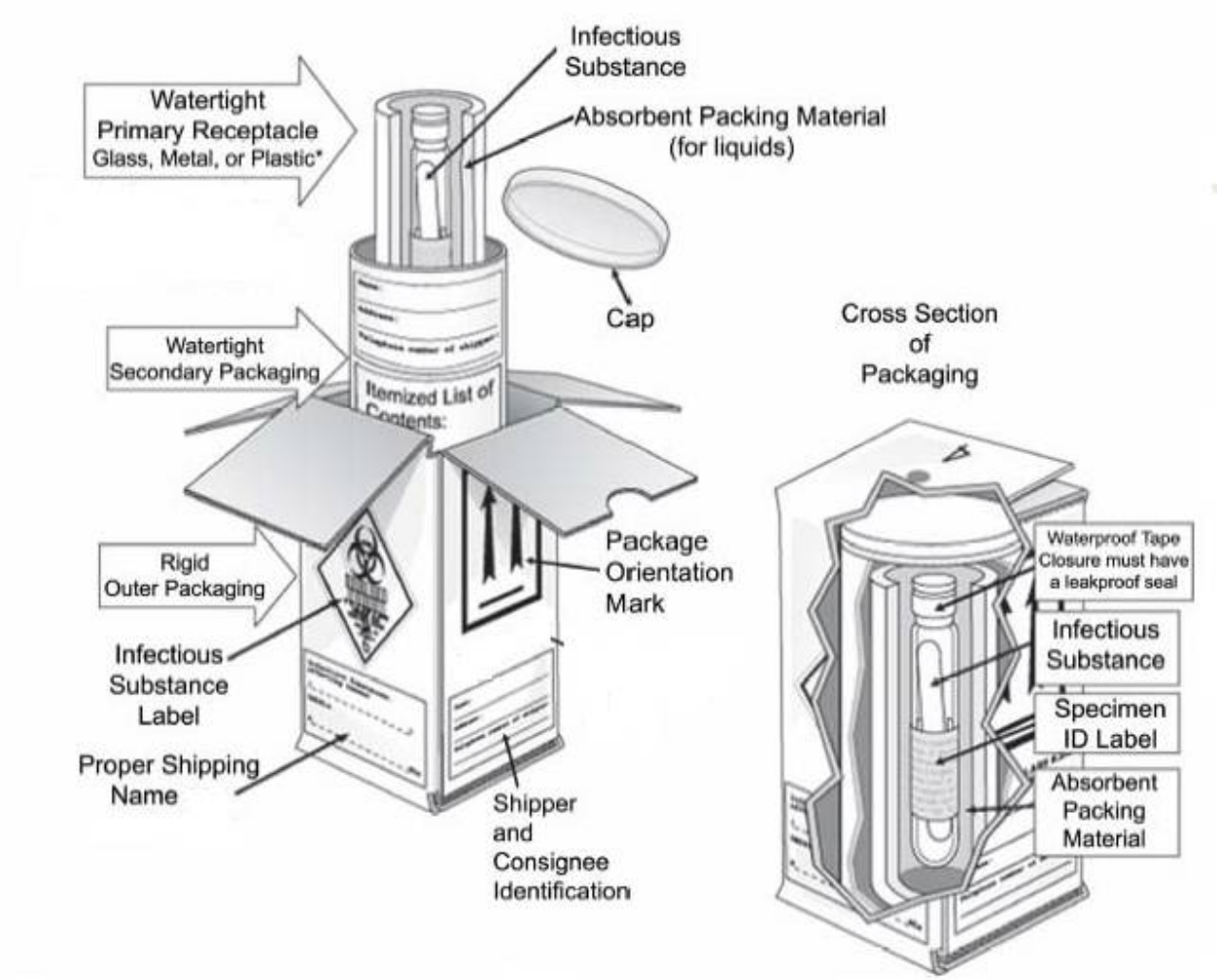


Fig 7.1. Triple packaging system

For surface transport there is no maximum quantity per package. For air transport the limits per package are as follows:

Category A

- 50 ml or 50 g for passenger aircraft
- 4 litres or 4 kg for cargo aircraft.

Category B

- 4 litres or 4 kg for passenger aircraft
- 1 litres per primary container for cargo aircraft.

7.3.2 Marking of packages

All markings on packages shall be placed in such a way that they are clearly visible and not covered by any other label or marking. Each package shall display the following information on the outer packaging.

- the shipper's (sender's, consignor's) name and address
- the telephone number of a responsible person, knowledgeable about the shipment
- the receiver's (consignee's) name and address
- Proper shipping name ("INFECTIOUS SUBSTANCE, AFFECTING HUMANS" or "INFECTIOUS SUBSTANCE, AFFECTING ANIMALS only or BIOLOGICAL SUBSTANCE CATEGORY B", as appropriate). Technical names need not be shown on the package.
- Temperature storage requirements (optional)
- When dry ice or liquid nitrogen is used: the technical name of the refrigerant and the net quantity.
- Any primary receptacle with a capacity of more than 50 ml shall be oriented in the outer packaging so that the closures are upwards. Orientation labels ("UP" arrows) shall be affixed to two opposite sides of the outer packaging.

7.3.3 Labelling

Hazard labels should be placed in the form of a square set at an angle of 45° (diamond- shaped)
Minimum dimensions: 100 × 100 mm (for small packages: 50 × 50 mm)
No. of labels per package: 1
Colour: Black and white
The words "INFECTIOUS SUBSTANCE" with the statement "In case of damage or leakage immediately notify a Public Health Authority" should be shown.

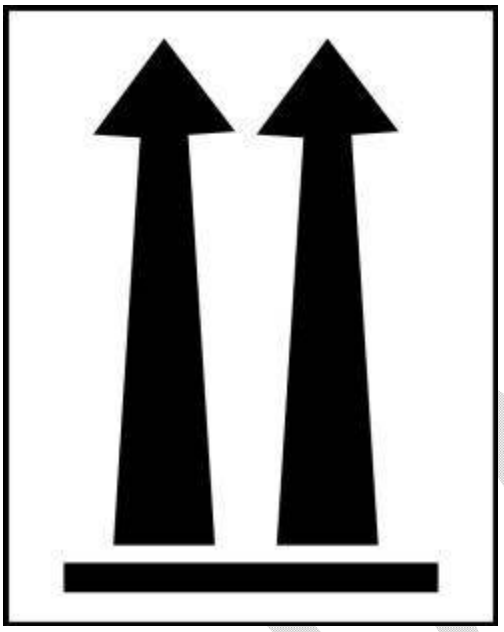
Orientation label

Minimum dimensions: 74 × 105 mm

No. per package: 2 on opposite sides

Colour: Black and white or red and white

The words “THIS SIDE UP” or “THIS END UP” may also be displayed on the top cover of the package.



The specimen carriers and ice packs can be reused after disinfection (Maintenance of transit temperature 4-8°C

7.3.4 Precautions

- Before transport, notify the receiving laboratory of all shipping and specimen details in advance of specimen arrival.
- Securely fasten transport boxes in the transport vehicle.
- Keep a spill kit in the vehicle containing absorbent materials, chlorine disinfectant, heavy-duty reusable gloves, mask, and apron and leak proof waste disposal container.
- Keep extra refrigerant in the vehicle (minimum of 4 ice packs will maintain refrigeration for 2-3 days) in case of delay in the travel schedule so that the cold chain is maintained.
- Avoid extensive vibration of specimens, such as that encountered when traveling for long periods over rough roads as this can hemolyse specimens, rendering them useless. If possible, separate the serum from clotted blood specimens before transport.

Chapter 8
Biosecurity in laboratories

8.1 Biosecurity

The term “biosecurity” refers to the protection of microbial agents from loss, theft, diversion or intentional misuse. The objective of biosecurity is to prevent loss, theft or misuse of microorganisms, biological materials, and research related information. This is accomplished by limiting access to facilities, research materials and information. While the objectives are different, biosafety and biosecurity measures are usually complementary while at times may conflicts.

8.2. Biosecurity and Biosafety

Biosafety and laboratory biosecurity are usually complementary and the implementation of specific biosafety activities already covers some biosecurity aspects. Biosafety is achieved by implementing various degrees of laboratory control and containment, through laboratory design and access restrictions, personnel expertise and training, use of containment equipment, and safe methods of managing infectious materials in a laboratory setting. The systematic use of appropriate biosafety principles and practices reduces the risk of accidental exposure and paves the way for reducing the risks of loss, theft or misuse of biological material. Laboratory biosecurity should be built upon a firm foundation of good laboratory biosafety.

Biosafety and biosecurity programs share common components. Both are based upon risk assessment and management methodology; personnel expertise and responsibility; control and accountability for research materials including microorganisms and culture stocks; access control elements; material transfer documentation; training; emergency planning; and program management.

Biosafety and biosecurity risk assessments are performed to determine the appropriate levels of controls within each program. Biosafety looks at appropriate laboratory procedures and practices necessary to prevent exposures and occupationally-acquired infections, while biosecurity addresses procedures and practices to ensure that biological materials and relevant sensitive information remain secure.

Both programs assess personnel qualifications. The biosafety program ensures that staff are qualified to perform their jobs safely through training and documentation of technical expertise. Staff must exhibit the appropriate level of professional responsibility for management of research materials by adherence to appropriate materials management

procedures. Biosafety practices require laboratory access to be limited when work is in progress. Biosecurity practices ensure that access to the laboratory facility and biological materials are limited and controlled as necessary. An inventory or material management process for control and tracking of biological stocks or other sensitive materials is also a component of both programs. For biosafety, the shipment of infectious biological materials must adhere to safe packaging, containment and appropriate transport procedures, while biosecurity ensures that transfers are controlled, tracked and documented with the potential risks. Both programs must engage laboratory personnel in the development of practices and procedures that fulfill the biosafety and biosecurity program objectives but that do not hinder research or clinical/diagnostic activities. The success of both of these programs hinges on a laboratory culture that understands and accepts the rationale for biosafety and biosecurity programs and the corresponding management oversight.

In some cases, biosecurity practices may conflict with biosafety practices, requiring personnel and management to devise policies that accommodate both sets of objectives. For example, signage may present a conflict between the two programs. Standard biosafety practice requires that signage be posted on laboratory doors to alert people to the hazards that may be present within the laboratory. The biohazard sign normally includes the name of the agent, specific hazards associated with the use or handling of the agent and contact information for the investigator. These practices may conflict with security objectives. Therefore, biosafety and biosecurity considerations must be balanced and proportional to the identified risks when developing institutional policies.

Designing a biosecurity program requires a familiarity with microbiology and the materials that require protection. Protecting pathogens and other sensitive biological materials while preserving the free exchange of research materials and information may present significant institutional challenges.

8.3. Valuable Biological Materials (VBM)

Valuable biological materials (VBM) are biological materials that require specific protective and monitoring measures in laboratories to protect the population from their potential to cause harm and/or their economic and historical (archival) value. Although all materials of a biological nature may fall within the definition of VBM, in fact not all VBM warrant exceptional protective measures or strict accounting. The classification of biological materials as VBM is the responsibility of their caretakers (laboratory managers and scientists) who should be able to address and define the level of protection required.

Laboratory biosecurity measures should be based on a comprehensive programme of accountability for VBM that includes:

a. regularly updated inventories with storage locations,
b. identification and selection of personnel with access,
c. plans of use of VBM,
d. clearance and approval processes,
e. documentation of internal and external transfers within and between facilities, and of any
f. inactivation and/or disposal of the material.

Likewise, institutional laboratory biosecurity protocols should include how to handle breaches or near-breaches in laboratory biosecurity including:

1. incident notification,
2. reporting protocols,
3. investigation reports,
4. recommendations and remedies, and
5. oversight and guidance through the Biosafety Committee.

The protocols should also include how to handle discrepancies in inventory results, and describe the specific training to be offered, and the training that personnel should be required to follow. The involvement, roles and responsibilities of public health and security authorities in the event of a security breach should also be clearly defined.

In many biological laboratories, only a small subset of VBM may be of high enough value or potential consequence to require detailed accountability or audit measures and substantial economic investment. However, laboratory biosecurity measures should not hamper the ability to work with, share and use of them.

8.4. Laboratory biosecurity programme

A specific laboratory biosecurity programme, managing the identified biorisks, should be prepared and designed for each facility according to its specific requirements, to the type of laboratory work conducted, and to local and geographical conditions. A comprehensive laboratory biosecurity programme involves:

1. identification of VBM
2. associated agent-based microbiological risk assessment and laboratory biosecurity risk assessment
3. bioethical and scientific analysis of research projects before they are authorized
4. allocation of responsibilities and authorities among staff and facility managers
5. communication between parties involved

6. development of and training on emergency plans; and
7. tailored biosecurity training for employees of the facility and for external first responders.

All these steps should be the result of a transparent and documented reasoning process that carefully evaluates the impact of biorisk management breaches, and prepares and plans for worst-case scenarios. Individual components of this programme are described below.

8.4.1. Laboratory biosecurity risk assessment

- Assessment of the suitability of personnel, training and adherence to VBM protection procedures are tools that may be used.
- It is important that these biorisk assessment efforts be regularly re-evaluated in an ongoing programme
- Assessment timing and scope, describing situations requiring a risk assessment to be carried out or an existing assessment to be re-evaluated, should also be clearly defined and adhered to.
- A competent scientific manager should be responsible for ensuring that appropriate risk assessments for research projects have been performed and cleared, and all records thereof are securely kept; that work is performed according to plan or only with authorized deviations from original plans; that management systems, procedures and records are properly maintained
- Collaboration between these different stakeholders and proactive clarification of their roles, responsibilities and authorities should help in case of emergencies.

8.4.2. Responsibility for VBM

Laboratory biosecurity should mainly be based on:

- control and accountability for VBM
- defining their storage location
- describing and scrutinizing their use; identifying personnel (and visitors) who should be granted access to them
- documenting their transfer
- certifying their inactivation and disposal, and
- sharing this information with appropriate counterparts within the facility.

Laboratory biosecurity measures should be adapted to the needs of the institutions or facilities adopting them. Their identification should be the result of a biosecurity risk

assessment that includes input from scientific personnel and laboratory management, biosafety officers, maintenance staff, IT staff, administrators and law-enforcement representatives

The facility should establish a clear working relationship with the local law enforcement agency to provide a response to security incidents on-site. Regular on-site training and orientation for the local law-enforcement agency is also recommended.

At facility level, it is recommended that the ultimate responsibility for VBM should lie with the laboratory/facility manager or director, who should be responsible for providing the appropriate conditions to minimize breaches in biosafety and laboratory biosecurity.

At international level, national authorities should be ultimately responsible for breaches in biosafety and laboratory biosecurity that may be at the origin of public health emergencies of international concern

8.4.3. Elements of Laboratory Biosecurity Plan

Laboratory biosecurity should specifically address the policies and procedures associated with physical biosecurity, staff security, transportation security, material control and information security. It should also include emergency response protocols that address security-related issues, such as specific instructions concerning when outside responders may be called (fire brigade, emergency medical personnel or security personnel), including the protocol to follow once on-site and the scope of authority of all parties involved. It is important for the laboratory security plan to anticipate the most likely situations that would require exceptional access. Just as training is essential for good biosafety practices, it is also essential to train for good biosecurity practices, particularly in emergency situations. Hence regular training of all personnel on security policies and procedures helps ensure correct implementation.

a. **Securing laboratory equipment**- safeguarding laboratory equipment from unauthorized access, misuse or removal is an important Biorisk management aspect of laboratory biosecurity that should also be addressed.
b. **Physical biosecurity** - Physical biosecurity, comprises of:
    a. Engineering, structural and security personnel elements intended to select, control and document access to laboratories and to the materials they contain, and to limit improper removal of relevant biological material and equipment.
    b. Access controls are used to limit access to restricted areas to individuals who have proper authorization and to keep track of traffic in and out of these areas.

c. **Personnel management** - Personnel management procedures must include the following:

i. Must define roles, responsibilities and authorities of laboratory personnel who need to handle, use, store, transfer and/or transport VBM, and the manner in which the organization ensures that individuals are appropriate for the positions they hold.

ii. Clearly describe and document the training, experience, competency and suitability requirements for individuals who have access to VBM, ensuring that members of the workforce have appropriate personal and technical qualifications and skills.

iii. Documented procedures for the recruitment of personnel should be clearly established and followed.

iv. The professional and bioethical eligibility and suitability for working with VBM of all personnel who have regular authorized access to sensitive materials is also central to effective laboratory biosecurity risk management.

v. A mechanism should be developed to ensure that the integrity of the facility will not be compromised through the absence of key individuals.

vi. Procedures and training for visitors, contractors, subcontractors, suppliers, cleaning and maintenance staff must also be addressed

d. **Information security**

i. Information security establishes prudent policies for handling sensitive details on VBM. Examples of sensitive information may include laboratory security plans and inventories, and storage locations of VBM. Information security should ensure that the required and appropriate level of confidentiality is preserved by the system that is used to acquire, store, manipulate and manage information.

ii. Higher the level of risk associated with the VBM the institution holds, the greater protection the information associated with the security system will require. Overdoing or exaggerating the sensitivity or level of suspicion can have unintended negative repercussions. This is a difficult process which may require careful consideration and reflection.

iii. Therefore laboratory management and relevant authorities should develop appropriate policies that govern the marking and handling of information and how that information is gathered, maintained, distributed, documented, accessed, shared and stored within the facility and with appropriate counterparts

8.4.4. Management of laboratory biosecurity activities

- Laboratory biosecurity activities should be established with clear and consistent policies and guidance.
- These activities should be integrated into the overall policies and administrative procedures of the facility.
- Managers are responsible for ensuring that biosecurity plans and incident response plans are enforced and revised as needed.
- Re-evaluation is a necessary and ongoing process since it is unlikely that the range of VBM and threats at any given institution will remain static.
- Biosecurity programme managers should also conduct biosecurity programme audits (assessments), provide remedial strategies for identified vulnerabilities and gaps, and ensure that the facility's threat and risk assessment is regularly reviewed and updated.
- Training and familiarization concerning the objectives and requirements of laboratory biosecurity activities should be ongoing

8.5. Training

Laboratory biosecurity training, complementary to laboratory biosafety training and commensurate with the roles, responsibilities and authorities of staff, should be provided to all those working at a facility, including maintenance and cleaning personnel, and to external first-responders and responsible staff involved in ensuring the security of the laboratory facility. Such training should:

- help understand the need for protection of VBM and equipment and rationale for the laboratory biosecurity measures adopted,
- include a review of relevant national policies and institution-specific procedures.
- provide procedures describing the security roles, responsibilities and authority of personnel in the event of emergencies or security breaches details of security risks judged not significant enough to warrant protection measures.

Training should not be offered regularly and taken recurrently. It should represent an opportunity for employees to refresh their memories and to learn about new developments and advances in different areas. Training is also important in providing occasions for discussions and bonding among staff members, and in strengthening of the team spirit among members of an institution.

8.6. Summary of Laboratory biosecurity activities

Laboratory biosecurity activities are the ultimate responsibility of laboratory directors whose tasks should include the ability to demonstrate that risks are appropriately managed, biorisk management programmes may be divided into seven main components:

1. Identify VBM that require protection on the basis of regularly performed biorisk assessments.
2. Establish clear guidance, roles, responsibilities and authorities for those who work with or have access to VBM and to the facilities that contain them.
3. Promote a culture of awareness, shared sense of responsibility, ethics, and respect of codes of conduct within the international life science community.
4. Develop policies that do not hinder the efficient sharing of reference materials and scientific data, clinical and epidemiological specimens and related information, and that do not impede the conduct of legitimate research.
5. Strengthen collaboration between the scientific, technical and security sectors.
6. Provide appropriate training to employees of laboratory facilities.
7. Strengthen emergency response and recovery plans on the assumption that biorisk management systems can only minimize, but never really eliminate, every conceivable threat.

Furthermore, the commitment to constantly improve biorisk management performance for a facility and its operation through attainable goal-setting and actual goal-achieving should be encouraged and acknowledged at all levels.

8.7. The biorisk management approach

Laboratory biorisk management is a system or process to control safety and security risks associated with the handling or storage and disposal of biological agents and toxins in laboratories and facilities. It is the analysis of ways and development of strategies to minimize the likelihood of the occurrence of biorisks. The management of biorisk places responsibility on the facility and its manager (director) to demonstrate that appropriate and valid biorisk reduction (minimization) procedures have been established and are implemented. One of the goals of the biorisk management approach is to develop a comprehensive laboratory biosafety and biosecurity culture, allowing biosafety and biosecurity to become part of the daily routine of a laboratory, improving the overall level of working conditions, and pushing for expected good laboratory management.

Some of the key factors in establishing and implementing a successful biorisk management system include:

- Commitment by top management:

- providing adequate resources, prioritization and communication of biosafety and biosecurity policy;
- integrating of biorisk management throughout the organization;
- Identifying opportunities for improvement and prevention, determining root causes and preventing recurrence.

- Focus on continual improvement:
  - making continual improvement an objective for every individual in the organization;
  - using periodic assessment against established risk-criteria to identify areas for potential improvement;
  - continually improving the effectiveness and efficiency of processes;
  - promoting prevention activities;
  - providing personnel in the organization with appropriate education and training including the methods and tools of continual improvement;
  - establishing measures and goals for improvement;
  - recognizing improvement.

8.8. Bioterrorism

Bioterrorism (also known as biological terrorism) is the intentional use of a biological agent or derivative of such an agent to inflict harm or death in people, animals, or plants.

8.8.1. Bioterrorism Agents

The agents used to cause bioterrorism are Bioterrorism Agents. These agents are typically found in nature, they could be possibly modified to increase their ability to cause disease, make them resistant to current medicines, or to increase their ability to be spread into the environment. They can be spread through the air, water, or in food.

There are a broad range of potential bioterrorism agents, including bacteria, viruses, and toxins (of microbial, plant, or animal origin). Common characteristics of this diverse group of agents include (1) the ability to be dispersed in the form of aerosols which can penetrate the distal bronchioles; (2) the ability to deliver these aerosols with simple technology; (3) the feasibility of these agents, if delivered from a line source (e.g., an airplane) upwind from the target, to infect large numbers of the population; and (4) the ability to spread infection, disease, panic, and fear. The most efficient method of delivering biological agents is thought to be the air-borne route, with agents dispersed in aerosols. Wide dissemination of infectious agents and even toxins can be achieved with this method. Low-cost, easily obtainable equipment (as employed in the agricultural industry) can be used to produce aerosols with particle sizes of 1 to 10 μm. Under

ideal conditions these particles may remain suspended for hours and are sufficiently small to make their way into the distal bronchioles and terminal alveoli after inhalation. Other methods of dissemination include oral (intentional contamination of food/water supply), percutaneous, infected animal vector (e.g., release of infected fleas), and human-to-human spread (individual infected with communicable disease walking among a crowd of healthy people).

8.8.2. Categories of Bioterrorism Agents

Bioterrorism agents can be separated into three categories (CDC, US), depending on how easily they can be spread and the severity of illness or death they cause. Category A agents are considered the highest risk and Category C agents are those that are considered emerging threats for disease.

Category A

These high-priority agents include organisms or toxins that pose the highest risk to the public and national security because:

- They can be easily spread or transmitted from person to person
- They result in high death rates and have the potential for major public health impact
- They might cause public panic and social disruption
- They require special action for public health preparedness.

Category B

These agents are the second highest priority because:

- They are moderately easy to spread
- They result in moderate illness rates and low death rates
- They require specific enhancements of CDC's laboratory capacity and enhanced disease monitoring.

Category C

These third highest priority agents include emerging pathogens that could be engineered for mass spread in the future because:

- They are easily available
- They are easily produced and spread
- They have potential for high morbidity and mortality rates and major health impact.

8.8.3. Identifying a Bioterrorism attack

Similar to an outbreak of an emerging pathogen, in a bioterrorism attack it will be imperative to establish the causative agent as quickly as possible. Treatment, prophylaxis, and control measures all depend on the causative agent. Timely detection and response is very important because patients with diseases caused by some of these agents can progress to death very

rapidly without appropriate treatment. For example, untreated pneumonic plague usually progresses to death within 36 to 72 hours. Of key importance is the infectious disease expertise of the investigating physician/epidemiologist in deciding which, if any, isolation precautions should be implemented while awaiting confirmatory diagnosis. Agent identification and patient diagnosis will depend a great deal on the effectiveness of the passive surveillance system used by the facility or agency. If clinicians have maintained a high index of suspicion and have a good knowledge foundation regarding the potential diseases that could be involved in an infectious disease disaster, it is more likely that the event/outbreak will be rapidly identified.

Bioterrorism agents are highly infectious and all guidelines for biosafety should be strictly followed. The specimen should be sent through courier on urgent basis to designated laboratory with appropriate transport instructions. Laboratories must have a step wise action plan prepared beforehand to cope up with any possible bioterrorism attack which must include preparedness to handle large number of specimens & transportations. They need to upgrade and develop rapid techniques for detection of diseases which they can handle.

When to suspect that disease outbreak is due to bioterrorism

Clues that may signal a bioterrorism attack are:

- Single case of disease caused by an uncommon agent (smallpox, unusual viral haemorrhagic fever etc.)
- Unusual, atypical, genetically engineered strains of an agent (or antibiotic resistance patterns)
- Disease with an unusual geographic or seasonal distribution
- Stable endemic disease with an unexplained increase in incidence
- Several unexplained or unusual diseases coexisting in the same patient without any explanation
- Unusual illness that affects a large diverse population or age group (e.g. outbreak of measles like rash in adults)
- Similar genetic type among agents isolated from temporally or spatially distinct sources
- Simultaneous clusters of similar illness in non-contiguous areas, domestic or foreign
- Large numbers of cases of unexplained disease or deaths

The laboratory clues suggestive of deliberate dissemination of a biologic agent are meaningful only in the context of a complete epidemiologic investigation. The clues listed above suggest that a cluster of cases is unusual from a public health perspective. Combination of clues, especially those that link clinical information with the epidemiologic features such as an uncommon agent isolated from large numbers of patients across the country, or an

unexplained increase in the incidence of Pneumonic plague- should increase the index of suspicion that an event may be due to bioterrorism. Epidemiologic judgement, like clinical judgement, will be important for determining what is unusual enough to warrant a concern regarding bioterrorism.

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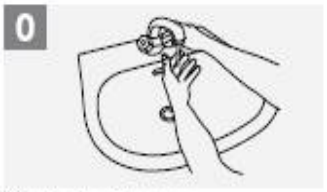
Annexure 1

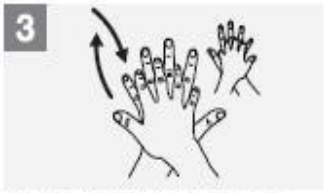
STEPS FOR GOOD HANDWASHING



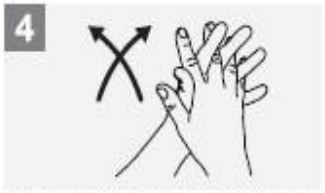
Wet hands with water;

Apply enough soap to cover all hand surfaces;

Rub hands palm to palm;



Right palm over left dorsum with interlaced fingers and vice versa;



Palm to palm with fingers interlaced;

Backs of fingers to opposing palms with fingers interlocked;

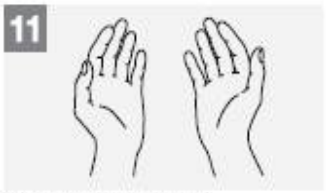
Rotational rubbing of left thumb clasped in right palm and vice versa;

Rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice versa;

Rinse hands with water;

Dry hands thoroughly with a single use towel;

Use towel to turn off faucet;



Your hands are now safe.

Annexure 2

Biomedical Waste Management Rules 2016

Duties of Occupier

1. Take all necessary steps to ensure that bio-medical waste is handled without any adverse effect to human health and the environment and in accordance with these rules;
2. Make a provision within the premises for a safe, ventilated and secured location for storage of segregated biomedical waste in coloured bags or containers in the manner as specified in Schedule I, to ensure that there shall be no secondary handling, pilferage of recyclables or inadvertent scattering or spillage by animals and the bio-medical waste from such place or premises shall be directly transported in the manner as prescribed in these rules to the common bio-medical waste treatment facility or for the appropriate treatment and disposal, as the case may be, in the manner as prescribed in Schedule I (Annexure 3)
3. Pre-treat the laboratory waste, microbiological waste, blood samples and blood bags through disinfection or sterilisation on-site in the manner as prescribed by the World Health Organisation (WHO) or National AIDs Control Organisation (NACO) guidelines and then sent to the common bio-medical waste treatment facility for final disposal;
4. Phase out use of chlorinated plastic bags, gloves and blood bags within two years from the date of notification of these rules;
5. Dispose of solid waste other than bio-medical waste in accordance with the provisions of respective waste management rules made under the relevant laws and amended from time to time;
6. Not to give treated bio-medical waste with municipal solid waste;
7. Provide training to all its health care workers and others, involved in handling of bio medical waste at the time of induction and thereafter at least once every year and the details of training programmes conducted, number of personnel trained and number of personnel not undergone any training shall be provided in the Annual Report;
8. Immunise all its health care workers and others, involved in handling of bio-medical waste for protection against diseases including Hepatitis B and Tetanus that are likely to be transmitted by handling of bio-medical waste, in the manner as prescribed in the National Immunisation Policy or the guidelines of the Ministry of Health and Family Welfare issued from time to time;
9. Establish a Bar- Code System for bags or containers containing bio-medical waste to be sent out of the premises or place for any purpose within one year from the date of the notification of these rules;
10. Ensure segregation of liquid chemical waste at source and ensure pre-treatment or neutralisation prior to mixing with other effluent generated from health care facilities;
11. Ensure treatment and disposal of liquid waste in accordance with the Water (Prevention and Control of Pollution) Act, 1974 (6 of 1974);

12. Ensure occupational safety of all its health care workers and others involved in handling of biomedical waste by providing appropriate and adequate personal protective equipment;
13. Conduct health check up at the time of induction and at least once in a year for all its health care workers and others involved in handling of bio- medical waste and maintain the records for the same;
14. Maintain and update on day to day basis the bio-medical waste management register and display the monthly record on its website according to the bio-medical waste generated in terms of category and colour coding as specified in Schedule I;
15. Report major accidents including accidents caused by fire hazards, blasts during handling of biomedical waste and the remedial action taken and the records relevant thereto, (including nil report) in Form I to the prescribed authority **and also** along with the annual report;
16. Make available the annual report on its web-site and all the health care facilities shall make own website within two years from the date of notification of these rules;
17. Inform the prescribed authority immediately in case the operator of a facility does not collect the bio-medical waste within the intended time or as per the agreed time;
18. Establish a system to review and monitor the activities related to bio-medical waste management, either through an existing committee or by forming a new committee and the Committee shall meet once in every six months and the record of the minutes of the meetings of this committee shall be submitted along with the annual report to the prescribed authority and the healthcare establishments having less than thirty beds shall designate a qualified person to review and monitor the activities relating to bio-medical waste management within that establishment and submit the annual report;
19. Maintain all record for operation of incineration, hydro or autoclaving etc., for a period of five years;
20. Existing incinerators to achieve the standards for treatment and disposal of bio-medical waste as specified in Schedule II for retention time in secondary chamber and Dioxin and Furans within two years from the date of this notification.

Annexure 3

Biomedical Waste Management Rules 2016

SCHEDULE I

[See rules 3 (e), 4(b), 7(1), 7(2), 7(5), 7 (6) and 8(2)]

Part-1

Biomedical wastes categories and their segregation, collection, treatment, processing and disposal options

Category	Type of waste	Type of Bag or container to be used	Treatment and Disposable options
(1)	**(2)**	**(3)**	**(4)**
Yellow	(**a) Human Anatomical Waste:** Human tissues, organs, body parts and fetus below the viability period (as per the Medical Termination of Pregnancy Act 1971, amended from time to time).	Yellow coloured non-chlorinated plastic bags	Incineration or Plasma Pyrolysis or deep burial*
	(b)Animal Anatomical Waste : Experimental animal carcasses, body parts, organs, tissues, including the waste generated from animals used in experiments or testing in veterinary hospitals or colleges or animal houses.		
	(c) Soiled Waste: Items contaminated with blood, body fluids like dressings, plaster casts, cotton swabs and bags containing residual or discarded blood and blood components.		Incineration or Plasma Pyrolysis or deep burial* In absence of above facilities, autoclaving or micro-waving/ hydroclaving followed by

			shredding or mutilation or combination of sterilization and shredding. Treated waste to be sent for energy recovery.
	(d) Expired or Discarded Medicines: Pharmaceutical waste like antibiotics, cytotoxic drugs including all items contaminated with cytotoxic drugs along with glass or plastic ampoules, vials etc.	Yellow coloured non-chlorinated plastic bags or containers	Expired `cytotoxic drugs and items contaminated with cytotoxic drugs to be returned back to the manufacturer or supplier for incineration at temperature >1200 ^{0}C or to common bio-medical waste treatment facility or hazardous waste treatment, storage and disposal facility for incineration at >1200^{0}C Or Encapsulation or Plasma Pyrolysis at >1200^{0}C. All other discarded medicines shall be either sent back to manufacturer or disposed by incineration.
	(e) Chemical Waste: Chemicals used in production of biological and used or discarded disinfectants.	Yellow coloured containers or non-chlorinated plastic bags	Disposed of by incineration or Plasma Pyrolysis or Encapsulation in hazardous waste treatment, storage and disposal facility.
	(f) Chemical Liquid Waste :	Separate collection	After resource recovery, the chemical

	Liquid waste generated due to use of chemicals in production of biological and used or discarded disinfectants, Silver X-ray film developing liquid, discarded Formalin, infected secretions, aspirated body fluids, liquid from laboratories and floor washings, cleaning, house-keeping and disinfecting activities etc.	system leading to effluent treatment system	liquid waste shall be pre-treated before mixing with other wastewater. The combined discharge shall conform to the discharge norms given in Schedule-III.
	(g) Discarded linen, mattresses, beddings contaminated with blood or body fluid.	Non-chlorinated yellow plastic bags or suitable packing material	Non- chlorinated chemical disinfection followed by incineration or Plazma Pyrolysis or for energy recovery. In absence of above facilities, shredding or mutilation or combination of sterilization and shredding. Treated waste to be sent for energy recovery or incineration or Plazma Pyrolysis.
	(h) Microbiology, Biotechnology and other clinical laboratory waste: Blood bags, Laboratory cultures, stocks or specimens of micro-organisms, live or	Autoclave safe plastic bags or containers	Pre-treat to sterilize with non-chlorinated chemicals on-site as per National AIDS Control Organisation or World Health Organisation guidelines thereafter for Incineration.

	attenuated vaccines, human and animal cell cultures used in research, industrial laboratories, production of biological, residual toxins, dishes and devices used for cultures.		
Red	**Contaminated Waste (Recyclable)** (a) Wastes generated from disposable items such as tubing, bottles, intravenous tubes and sets, catheters, urine bags, syringes (without needles and fixed needle syringes) and vaccutainers with their needles cut) and gloves.	Red coloured non-chlorinated plastic bags or containers	Autoclaving or micro-waving/ hydroclaving followed by shredding or mutilation or combination of sterilization and shredding. Treated waste to be sent to registered or authorized recyclers or for energy recovery or plastics to diesel or fuel oil or for road making, whichever is possible. Plastic waste should not be sent to landfill sites.
White (Translucent)	**Waste sharps including Metals:** Needles, syringes with fixed needles, needles from needle tip cutter or burner, scalpels, blades, or any other contaminated sharp object that may cause puncture and cuts. This includes both used, discarded and	Puncture proof, Leak proof, tamper proof containers	Autoclaving or Dry Heat Sterilization followed by shredding or mutilation or encapsulation in metal container or cement concrete; combination of shredding cum autoclaving; and sent for

	contaminated metal sharps		final disposal to iron foundries (having consent to operate from the State Pollution Control Boards or Pollution Control Committees) or sanitary landfill or designated concrete waste sharp pit.
Blue	**(a) Glassware:** Broken or discarded and contaminated glass including medicine vials and ampoules except those contaminated with cytotoxic wastes.	Cardboard boxes with blue colored marking	Disinfection (by soaking the washed glass waste after cleaning with detergent and Sodium Hypochlorite treatment) or through autoclaving or microwaving or hydroclaving and then sent for recycling.
	(b) Metallic Body Implants	Cardboard boxes with blue colored marking	

***Disposal by deep burial is permitted only in rural or remote areas where there is no access to common bio-medical waste treatment facility. This will be carried out with prior approval from the prescribed authority and as per the Standards specified in Schedule-III. The deep burial facility shall be located as per the provisions and guidelines issued by Central Pollution Control Board from time to time.**

Part-2

1. All plastic bags shall be as per BIS standards as and when published, till then the prevailing Plastic Waste Management Rules shall be applicable.
2. Chemical treatment using at least 10% Sodium Hypochlorite having 30% residual chlorine for twenty minutes or any other equivalent chemical reagent that should demonstrate Log104 reduction efficiency for microorganisms as given in Schedule- III.

3. Mutilation or shredding must be to an extent to prevent unauthorized reuse.
4. There will be no chemical pre-treatment before incineration, except for microbiological, laboratory and highly infectious waste.
5. Incineration ash (ash from incineration of any bio-medical waste) shall be disposed through hazardous waste treatment, storage and disposal facility, if toxic or hazardous constituents are present beyond the prescribed limits as given in the Hazardous Waste (Management, Handling and Transboundary Movement) Rules, 2008 or as revised from time to time.
6. Dead Fetus below the viability period (as per the Medical Termination of Pregnancy Act 1971, amended from time to time) can be considered as human anatomical waste. Such waste should be handed over to the operator of common bio-medical waste treatment and disposal facility in yellow bag with a copy of the official Medical Termination of Pregnancy certificate from the Obstetrician or the Medical Superintendent of hospital or healthcare establishment.
7. Cytotoxic drug vials shall not be handed over to unauthorised person under any circumstances. These shall be sent back to the manufactures for necessary disposal at a single point. As a second option, these may be sent for incineration at common bio-medical waste treatment and disposal facility or TSDFs or plasma pyrolysis at temperature >1200 0C.
8. Residual or discarded chemical wastes, used or discarded disinfectants and chemical sludge can be disposed at hazardous waste treatment, storage and disposal facility. In such case, the waste should be sent to hazardous waste treatment, storage and disposal facility through operator of common bio-medical waste treatment and disposal facility only.
9. On-site pre-treatment of laboratory waste, microbiological waste, blood specimens, blood bags should be disinfected or sterilized as per the Guidelines of World Health Organisation or National AIDS Control Organisation and then given to the common bio-medical waste treatment and disposal facility.
10. Installation of in-house incinerator is not allowed. However in case there is no common biomedical facility nearby, the same may be installed by the occupier after taking authorisation from the State Pollution Control Board.
11. Syringes should be either mutilated or needles should be cut and or stored in tamper proof, leak proof and puncture proof containers for sharps storage. Wherever the occupier is not linked to a disposal facility it shall be the responsibility of the occupier to sterilize and dispose in the manner prescribed.

Bio-medical waste generated in households during healthcare activities shall be segregated as per these rules and handed over in separate bags or containers to municipal waste collectors. Urban Local Bodies shall have tie up with the common bio-medical waste treatment and disposal facility to pick up this waste from the Material Recovery Facility (MRF) or from the house hold directly, for final disposal in the manner as prescribed in this Schedule.

Annexure 4

Biomedical Waste Management Rules 2016

Duties of the operator of a common bio-medical waste treatment and disposal facility.

It shall be the duty of every operator to –

1. Take all necessary steps to ensure that the bio-medical waste collected from the occupier is transported, handled, stored, treated and disposed of, without any adverse effect to the human health and the environment, in accordance with these rules and guidelines issued by the Central Government or, as the case may be, the central pollution control board from time to time;
2. Ensure timely collection of bio-medical waste from the occupier as prescribed under these rules;
3. Establish bar coding and global positioning system for handling of bio- medical waste within one year;
4. Inform the prescribed authority immediately regarding the occupiers which are not handing over the segregated bio-medical waste in accordance with these rules;
5. Provide training for all its workers involved in handling of bio-medical waste at the time of induction and at least once a year thereafter;
6. Assist the occupier in training conducted by them for bio-medical waste management;
7. Undertake appropriate medical examination at the time of induction and at least once in a year and immunise all its workers involved in handling of bio-medical waste for protection against diseases, including Hepatitis B and Tetanus, that are likely to be transmitted while handling bio-medical waste and maintain the records for the same;
8. Ensure occupational safety of all its workers involved in handling of bio-medical waste by providing appropriate and adequate personal protective equipment;
9. Report major accidents including accidents caused by fire hazards, blasts during handling of bio-medical waste and the remedial action taken and the records relevant thereto, (including nil report) in Form I (refer to rules) to the prescribed authority and also along with the annual report;
10. Maintain a log book for each of its treatment equipment according to weight of batch; categories of waste treated; time, date and duration of treatment cycle and total hours of operation;
11. Allow occupier , who are giving waste for treatment to the operator, to see whether the treatment is carried out as per the rules;

12. Shall display details of authorisation, treatment, annual report etc. on its web-site; (m) after ensuring treatment by autoclaving or microwaving followed by mutilation or shredding, whichever is applicable, the recyclables from the treated bio-medical wastes such as plastics and glass, shall be given to recyclers having valid consent or authorisation or registration from the respective State Pollution Control Board or Pollution Control Committee;
13. Supply non-chlorinated plastic coloured bags to the occupier on chargeable basis, if required;
14. Common bio-medical waste treatment facility shall ensure collection of biomedical waste on holidays also;
15. Maintain all record for operation of incineration, hydro or autoclaving for a period of five years; and
16. Upgrade existing incinerators to achieve the standards for retention time in secondary chamber and Dioxin and Furans within two years from the date of this notification.

Annexure 5

SCHEDULE II

[See rule 4(t), 7(1) and 7(6)]

STANDARDS FOR TREATMENT AND DISPOSAL OF BIO-MEDICALWASTES

1. STANDARDS FOR INCINERATION.-

All incinerators shall meet the following operating and emission standards

A. Operating Standards

1. Combustion efficiency (CE) shall be at least 99.00%.
2. The Combustion efficiency is computed as follows:

$$C.E. = \frac{\%C02}{\%C02 + \%\ CO} \times 100$$

3. The temperature of the primary chamber shall be a minimum of 800 0C and the secondary chamber shall be minimum of 10500C + or - 500C.
4. The secondary chamber gas residence time shall be at least two seconds.

B. Emission Standards

Sl. No.	**Parameter**	**Standards**	
(1)	**(2)**	**(3)**	**(4)**
		Limiting concentration in mg Nm^3 unless stated	**Sampling Duration in minutes, unless stated**
1.	Particulate matter	50	30 or $1NM^3$ of specimen volume, whichever is more
2.	Nitrogen Oxides NO	400	30 for online sampling or grab

	and NO_2 expressed asNO_2		Specimen
3.	HCl	50	30 or 1NM^3 of specimen volume, whichever is more
4.	Total Dioxins and Furans	0.1ngTEQ/Nm^3 (at 11% O2)	8 hours or 5NM^3 of specimen volume, whichever is more
5.	Hg and its compounds	0.05	2 hours or 1NM^3 of specimen volume, whichever is more

C. **Stack Height**:

Minimum stack height shall be 30 meters above the ground and hall be attached with the necessary monitoring facilities as per requirement of monitoring of 'general parameters' as notified under the Environment (Protection) Act, 1986 and in accordance with the Central Pollution Control Board Guidelines of Emission Regulation Part-III.

Note:

1. The existing incinerators shall comply with the above within a period of two years from the date of the notification.
2. The existing incinerators shall comply with the standards for Dioxins and Furans of 0.1ngTEQ/Nm3, as given below within two years from the date of commencement of these rules.
3. All upcoming common bio-medical waste treatment facilities having incineration facility or captive incinerator shall comply with standards for Dioxins and Furans.
4. The existing secondary combustion chambers of the incinerator and the pollution control devices shall be suitably retrofitted, if necessary, to achieve the emission limits.
5. Wastes to be incinerated shall not be chemically treated with any chlorinated disinfectants.
6. Ash from incineration of biomedical waste shall be disposed of at common hazardous waste treatment and disposal facility. However, it may be disposed of in municipal landfill, if the toxic metals in incineration ash are within the regulatory quantities as

defined under the Hazardous Waste (Management and Handling and Transboundary Movement) Rules, 2008 as amended from time to time.

7. Only low Sulphur fuel like Light Diesel Oil or Low Sulphur Heavy Stock or Diesel, Compressed
   Natural Gas, Liquefied Natural Gas or Liquefied Petroleum Gas shall be used as fuel in the
   incinerator.
8. The occupier or operator of a common bio-medical waste treatment facility shall monitor the stack gaseous emissions (under optimum capacity of the incinerator) once in three months through a laboratory approved under the Environment (Protection) Act, 1986 and record of such analysis results shall be maintained and submitted to the prescribed authority. In case of dioxins and furans, monitoring should be done once in a year.
9. The occupier or operator of the common bio-medical waste treatment facility shall install continuous emission monitoring system for the parameters as stipulated by State Pollution Control Board or Pollution Control Committees in authorisation and transmit the data real time to the servers at State Pollution Control Board or Pollution Control Committees and Central Pollution Control Board.
10. All monitored values shall be corrected to 11% Oxygen on dry basis.
11. Incinerators (combustion chambers) shall be operated with such temperature, retention time and turbulence, as to achieve Total Organic Carbon content in the slag and bottom ashes less than 3% or their loss on ignition shall be less than 5% of the dry weight.
12. The occupier or operator of a common bio-medical waste incinerator shall use combustion gas analyser to measure CO2, CO and O2.

2. Operating and Emission Standards for Disposal by Plasma Pyrolysis or Gasification:

A. Operating Standards:

All the operators of the Plasma Pyrolysis or Gasification **s**hall meet the following operating and emission standards:

1) Combustion Efficiency (CE) shall be at least 99.99%.
2) The Combustion Efficiency is computed as follows:

$$C.E = \frac{(\%CO_2)}{(\%\ CO_2 + \%\ CO)} \times 100$$

3) The temperature of the combustion chamber after plasma gasification shall be 1050 ± 50 o C with gas residence time of at least 2(two) second, with minimum 3 % Oxygen in the stack gas.
4) The Stack height should be minimum of 30 m above ground level and shall be attached with the necessary monitoring facilities as per requirement of monitoring of 'general parameters' as notified under the Environment (Protection) Act, 1986 and in accordance with the CPCB Guidelines of Emission Regulation Part-III.

B. Air Emission Standards and Air Pollution Control Measures

1. Emission standards for incinerator, notified at Sl No.1 above in this Schedule, and revised from time to time, shall be applicable for the Plasma Pyrolysis or Gasification also.
2. Suitably designed air pollution control devices shall be installed or retrofitted with the 'Plasma Pyrolysis or Gasification to achieve the above emission limits, if necessary.
3. Wastes to be treated using Plasma Pyrolysis or Gasification shall not be chemically treated with any chlorinated disinfectants and chlorinated plastics shall not be treated in the system.

D. Disposal of Ash Vitrified Material:

The ash or vitrified material generated from the 'Plasma Pyrolysis or Gasification shall be disposed off in accordance with the Hazardous Waste (Management, Handling and Transboundary Movement) Rules 2008 and revisions made thereafter in case the constituents exceed the limits prescribed under Schedule II of the said Rules or else in accordance with the provisions of the Environment (Protection) Act, 1986, whichever is applicable.

3. STANDARDS FOR AUTOCLAVING OF BIO-MEDICAL WASTE.-

The autoclave should be dedicated for the purposes of disinfecting and treating bio-medical waste.

(1) When operating a gravity flow autoclave, medical waste shall be subjected to:

a. a temperature of not less than 121° C and pressure of 15 pounds per square inch (psi) for an autoclave residence time of not less than 60 minutes; or
b. a temperature of not less than 135° C and a pressure of 31 psi for an autoclave residence time of not less than 45 minutes; or
c. a temperature of not less than 149° C and a pressure of 52 psi for an autoclave residence time of not less than 30 minutes.

(2) When operating a vacuum autoclave, medical waste shall be subjected to a minimum of three pre-vacuum pulse to purge the autoclave of all air. The air removed during the pre-vacuum, cycle should be decontaminated by means of HEPA and activated carbon filtration, steam treatment, or any other method to prevent release of pathogen. The waste shall be subjected to the following:

a. a temperature of not less than 121°C and pressure of 15 psi per an autoclave residence time of not less than 45 minutes; or
b. a temperature of not less than 135°C and a pressure of 31 psi for an autoclave residence time of not less than 30 minutes;

(3) Medical waste shall not be considered as properly treated unless the time, temperature and pressure indicators indicate that the required time, temperature and pressure were reached during the autoclave process. If for any reasons, time temperature or pressure indicator indicates that the required temperature, pressure or residence time was not reached, the entire load of medical waste must be autoclaved again until the proper temperature, pressure and residence time were achieved.

(4) **Recording of operational parameters:** Each autoclave shall have graphic or computer recording devices which will automatically and continuously monitor and record dates, time of day, load identification number and operating parameters throughout the entire length of the autoclave cycle.

(5) **Validation test for autoclave:** The validation test shall use four biological indicator strips, one shall be used as a control and left at room temperature, and three shall be placed in the approximate center of three containers with the waste. Personal protective equipment (gloves, face mask and coveralls) shall be used when opening containers for the purpose of placing the biological indicators. At least one of the containers with a biological indicator should be placed in the most difficult location for steam to penetrate, generally the bottom center of the waste pile. The occupier or operator shall conduct this test three consecutive times to define the minimum

operating conditions. The temperature, pressure and residence time at which all biological indicator vials or strips for three consecutive tests show complete inactivation of the spores shall define the minimum operating conditions for the autoclave. After determining the minimum temperature, pressure and residence time, the occupier or operator of a common biomedical waste treatment facility shall conduct this test once in three months and records in this regard shall be maintained.

(6) **Routine Test:** A chemical indicator strip or tape that changes colour when a certain temperature is reached can be used to verify that a specific temperature has been achieved. It may be necessary to use more than one strip over the waste package at different locations to ensure that the inner content of the package has been adequately autoclaved. The occupier or operator of a common bio medical waste treatment facility shall conduct this test during autoclaving of each batch and records in this regard shall be maintained.

(7) **Spore testing:** The autoclave should completely and consistently kill the approved biological indicator at the maximum design capacity of each autoclave unit. Biological indicator for autoclave shall be Geobacillusstearothermophilus spores using vials or spore Strips; with at least 1X106 spores. Under no circumstances will an autoclave have minimum operating parameters less than a residence time of 30 minutes, a temperature less than 121o C or a pressure less than 15 psi. The occupier or operator of a common bio medical waste treatment and disposal facility shall conduct this test at least once in every week and records in this regard shall be maintained.

4.STANDARDS OF MICROWAVING.-

1) Microwave treatment shall not be used for cytotoxic, hazardous or radioactive wastes, contaminated animal carcasses, body parts and large metal items.
2) The microwave system shall comply with the efficacy test or routine tests and a performance guarantee may be provided by the supplier before operation of the limit.
3) The microwave should completely and consistently kill the bacteria and other pathogenic

   organisms that are ensured by approved biological indicator at the maximum design capacity of each microwave unit. Biological indicators for microwave shall be Bacillus atrophaeusspores using vials or spore strips with at least 1 x 104sporesper detachable

strip. The biological indicator shall be placed with waste and exposed to same conditions as the waste during a normal treatment cycle.

5.STANDARDS FOR DEEP BURIAL.-

1) A pit or trench should be dug about two meters deep. It should be half filled with waste, then covered with lime within 50 cm of the surface, before filling the rest of the pit with soil.
2) It must be ensured that animals do not have any access to burial sites. Covers of galvanised iron or wire meshes may be used.
3) On each occasion, when wastes are added to the pit, a layer of 10 cm of soil shall be added to cover the wastes.
4) Burial must be performed under close and dedicated supervision.
5) The deep burial site should be relatively impermeable and no shallow well should be close to the site.
6) The pits should be distant from habitation, and located so as to ensure that no contamination occurs to surface water or ground water. The area should not be prone to flooding or erosion.
7) The location of the deep burial site shall be authorised by the prescribed authority.
8) The institution shall maintain a record of all pits used for deep burial.
9) The ground water table level should be a minimum of six meters below the lower level of deep burial pit.

6. STANDARDS FOR EFFICACY OF CHEMICAL DISINFECTION

Microbial inactivation efficacy is equated to "Log10 kill" which is defined as the difference between the logarithms of number of test microorganisms before and after chemical treatment. Chemical disinfection methods shall demonstrate a 4 Log10 reduction or greater for Bacillus Subtilis (ATCC 19659) in chemical treatment systems.

7. STANDARDS FOR DRY HEAT STERILIZATION

Waste sharps can be treated by dry heat sterilization at a temperature not less than 1850C, at least for a residence period of 150 minutes in each cycle, which sterilization period of 90 minutes. There should be automatic recording system to monitor operating parameters.

i. Validation test for Sharps sterilization unit

Waste sharps sterilization unit should completely and consistently kill the biological indicator Geobacillus Stearothermophillus or Bacillus Atropheausspoers using vials with at least log10 6 spores per ml. The test shall be carried out once in three months

ii. **Routine test**

A chemical indicator strip or tape that changes colour when a certain temperature is reached can be used to verify that a specific temperature has been achieved. It may be necessary to use more than one strip over the waste to ensure that the inner content of the sharps has been adequately disinfected. This test shall be performed once in week and records in this regard shall be maintained.

8. STANDARDS FOR LIQUID WASTE.-

1) The effluent generated or treated from the premises of occupier or operator of a common bio medical waste treatment and disposal facility, before discharge into the sewer should conform to the following limits

PARAMETERS	PERMISSIBLE LIMITS
pH	6.5-9.0
Suspended solids	100 mg/l
Oil and grease	10 mg/l
BOD	30 mg/l
COD	250 mg/l
Bio-assay test	90% survival of fish after 96 hours in 100% effluent.

2) Sludge from Effluent Treatment Plant shall be given to common bio-medical waste treatment facility for incineration or to hazardous waste treatment, storage and disposal facility for disposal.

Needle Stick / Sharp injury protocol for Blood Borne Pathogen

(To be filled by Supervisor)

1. Name and Full address of Hospital :_____
2. Name:_____Age/Sex_____Employment No.:_____
3. Section:_____Designation:_____
4. Date of reporting:_____ Date of injury: _____
5. Nature of injury : Needle prick (Solid needle / Hollow bore) / Sharp cut / Laceration / Splattered glass / Any other injury_____
6. Site & Size of injury :_____
7. How did the accident happen :_____
8. Immediate Action taken :
   a. Washing with soap and water — Yes / No — Duration:_____
   b. Reassurance — Yes / No
   c. First Aid — Yes / No
9. History of vaccination / prophylaxis : Yes / No
   a. Prior history of Hepatitis B Vaccine : Yes / No
   b. Documentation of Hepatitis. B vaccination : Available / Not available
   c. Schedule and date of Hepatitis. B vaccination : _____
   d. Anti Hepatitis B titer : Date: _____ Titer :_____
   e. Last tetanus toxoid with date : _____
10. Status of source at the time of injury :
    HIV : Positive / Negative / Not Known
    HBsAg : Positive / Negative / Not Known
    HCV : Positive / Negative / Not Known
    In an event of needle stick / sharp injury sample collected from source after counseling and consent: Yes / No
    Result :_____
11. Status of exposed person at the time of injury :
    a. HIV Antibody — Positive / Negative — By _____ test, dated _____
    b. HbsAg — Positive / Negative — By _____ test, dated _____
    c. HCV Antibody — Positive / Negative — By _____ test, dated _____
    Result of follow up testing up to 6 months_____
12. Action taken by Medical officer ,Referral date & time : _____
    a. First Aid given: Yes / No
    b. Tetanus vaccine given : Yes / No
    c. Post exposure prophylaxis for HIV : Yes / No (If Yes,Date)_____
       Basic /Expanded regimen with duration :_____
       Compliance: Yes / No
    d. Post exposure prophylaxis for Hepatitis B : Yes / No
       Hepatitis B immunoglobulin (HBIG): Yes / No (Date)_____
       Hepatitis B vaccine : Yes / No (Date)_____
13. Any other relevant information :_____

Signature of Officer in-charge **Signature of Supervisor**

Annexure 7

Indicative examples of infectious substances included in category A in any form unless otherwise indicated	
Proper Shipping Name	**Microorganism**
INFECTIOUS SUBSTANCE, AFFECTING HUMAN	Bacillus anthracis (cultures only) Brucella abortus (cultures only) Brucella melitensis (cultures only) Brucella suis (cultures only) Burkholderia mallei – Pseudomonas mallei – glanders (cultures only) Burkholderia pseudomallei – Pseudomonas pseudomallei (cultures only) Chlamydia psittaci – avian strains (cultures only) Clostridium botulinum (cultures only) Coccidioides immitis (cultures only) Coxiella burnetii (cultures only) Crimean-Congo haemorrhagic fever virus Dengue virus (cultures only) Eastern equine encephalitis virus (cultures only) Escherichia coli, verotoxigenic (cultures only)* Ebola virus Flexal virus Francisella tularensis (cultures only) Guanarito virus Hantaan virus Hantaviruses causing haemorrhagic fever with renal syndrome Hendra virus Hepatitis B virus (cultures only) Herpes B virus (cultures only) Human immunodeficiency virus (cultures only) Highly pathogenic avian influenza virus (cultures only) Japanese Encephalitis virus (cultures only) Junin virus Kyasanur Forest disease virus Lassa virus Machupo virus Marburg virus Monkeypox virus Mycobacterium tuberculosis (cultures only)* Nipah virus Omsk haemorrhagic fever virus

	Poliovirus (cultures only) Rabies virus (cultures only) Rickettsia prowazekii (cultures only) Rickettsia rickettsii (cultures only) Rift Valley fever virus (cultures only) Russian spring-summer encephalitis virus (cultures only) Sabia virus Shigella dysenteriae type 1 (cultures only)* Tick-borne encephalitis virus (cultures only) Variola virus Venezuelan equine encephalitis virus (cultures only) West Nile virus (cultures only) Yellow fever virus (cultures only) Yersinia pestis (cultures only)
INFECTIOUS SUBSTANCE, AFFECTING ANIMALS only	African swine fever virus (cultures only) Avian paramyxovirus Type 1 – Velogenic Newcastle disease virus (cultures only) Classical swine fever virus (cultures only) Foot and mouth disease virus (cultures only) Lumpy skin disease virus (cultures only) Mycoplasma mycoides – contagious bovine pleuropneumonia (cultures only) Peste des petits ruminants virus (cultures only) Rinderpest virus (cultures only) Sheep-pox virus (cultures only) Goatpox virus (cultures only) Swine vesicular disease virus (cultures only) Vesicular stomatitis virus (cultures only)

Note:

The table is not exhaustive. Infectious substances, including new or emerging pathogens, which do not appear in the table but which meet the same criteria shall be assigned to Category A. In addition, if there is doubt as to whether or not a substance meets the criteria it shall be included in Category A.

*For surface transport, when the cultures are intended for diagnostic or clinical purposes, they may be classified as infectious substances of Category B.

Annexure 8

List of Bioterrorism Agents/Diseases

Category A

Bacillus anthracis (anthrax)
Botulism (*Clostridium botulinum* toxin)
Yersinia pestis (plague)
Variola major (smallpox)
Francisella tularensis (tularemia)
Arenaviruses: Lassa, New World (Machupo, Junin, Guanarito and Sabia (Viral haemorrhagic fevers)
Bunyaviridae: Crimean Congo, Rift Valley (Viral haemorrhagic fevers)
Filoviridae: Ebola, Marburg (Viral haemorrhagic fevers)

Category B

Brucella species (Brucellosis)
Clostridium perfringens (Epsilon toxin)
Escherichia coli O157:H7 (*E. coli*)
Shigella (shigellosis)
Salmonella species (salmonellosis)(Food borne illness)
Salmonella Typhi (typhoid fever)
Burkholderia mallei (glanders)
Chlamydia psittaci (psittacosis)
Coxiella burnetii (Q fever)
Ricinus communis (castor beans) (Ricin toxin)
Staphylococcal (enterotoxin B)
Rickettsia prowazekii (typhus fever)
Vibrie cholera (Cholera) (water safety threats)
Cryptosporidium parvum (Water safety threats)

Category C

Nipah
Hanta
SARS
MERS coronavirus
Pandemic influenza

ABBREVIATIONS

BMW	Bio medical Waste
BSC	Biosafety Cabinet
BSL	Biosafety Level
CPCB	Central Pollution Control Board
GMT	Good Microbiological Techniques
HCW	Health Care Worker
HEPA	High Efficiency Particulate Air
LAI	Laboratory Associated Infections
NCDC	National Centre for Disease Control
PEP	Post Exposure Prophylaxis
PPE	Personal Protective Equipment
VBM	Valuable Biological Materials
WHO	World Health Organisation

GLOSSARY

Personal protective equipment- the equipment worn by health care workers in order to Safe guard themselves whenever dealing with infectious material.

Standard Precautions- Standard Precautions represent the minimum infection prevention measures that apply to all patient care, regardless of suspected or confirmed infection status of the patient, in any setting where healthcare is delivered. These evidence-based practices are designed to both protect healthcare personnel and prevent the spread of infections among patients. Standard Precautions replaces earlier guidance relating to Universal Precautions and Body Substance Isolation.

Biorisk - The probability or chance that a particular adverse event possibly leading to harm, will occur.

Biorisk assessment - The process to identify acceptable and unacceptable risks (embracing biosafety risks (risks of accidental infection) and laboratory biosecurity risks (risks of unauthorized access, loss, theft, misuse, diversion or intentional release)) and their potential consequences.

Biorisk management - The analysis of ways and development of strategies to minimize the likelihood of the occurrence of biorisks. The management of biorisk places responsibility on the facility and its manager (director) to demonstrate that appropriate and valid biorisk reduction (minimization) procedures have been established and are implemented. A biorisk management committee should be established to assist the facility director in identifying, developing and reaching biorisk management goals.

Hazard - A danger or source of danger; the potential to cause harm.

Laboratory biosecurity - Laboratory biosecurity describes the protection, control and accountability for valuable biological materials (VBM) within laboratories, in order to prevent their unauthorized access, loss, theft, misuse, diversion or intentional release.

Valuable biological materials (VBM) - Biological materials that require (according to their users, custodians, caretakers or regulators) administrative oversight, control, accountability, and specific protective and monitoring measures in laboratories to protect their economic and historical (archival) value, and/or the population from their potential to cause harm. VBM may include pathogens and toxins, as well as non-pathogenic organisms, vaccine strains, foods, genetically modified organisms (GMOs), cell components, genetic elements, and extraterrestrial samples.

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