Standard Operating Procedures for Laboratory Biosafety Biosecurity and Infection Control in Bangladesh

IEDCR

World Health Organization
Country Office for Bangladesh

Standard Operating Procedures for Laboratory Biosafety, Biosecurity and Infection Control in Bangladesh

Institute of Epidemiology, Disease Control and Research (IEDCR)
Directorate General of Health Services (DGHS)
Ministry of Health and Family Welfare
Government of the People's Republic of Bangladesh

Technical support:
World Health Organization (WHO) Country Office for Bangladesh

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1st Edition: 2015- 2016

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Foreword

Laboratory personnel handling clinical samples containing highly infectious agents are at high risk of contracting laboratory-acquired infection. This risk is particularly high for those working in microbiological laboratories. Laboratory-acquired infections involving contagious diseases have demonstrated the potential to spread beyond the laboratory into the general community at large. The situation in private and public clinical diagnostic laboratories that constitute an integral part of most hospitals in Bangladesh is unlikely to be any different.

Responsibility for biosafety exists at all levels and is shared throughout the Country. The IEDCR Biosafety Program was administered to establish procedures for the safe use of biohazards and for compliance with all applicable regulations. The all laboratory personnel who perform work with biohazards are the most important component of the biosafety program, as they must incorporate the biosafety requirements and safety precautions into all facets of their work.

The SOP's were developed by the laboratory experts of the country giving special emphasis on biosafety, biosecurity and infection control. The sample collection, sample handling, use of PPE and infection control during and after the laboratory procedures were given importance highlighting the emerging infectious diseases. These documents might support enormously in standard infection control procedures in the different level of laboratories in Bangladesh.

Contents

List of Abbreviations

BSC	-	Biological Safety Cabinet
BSL	-	Biological Safety Level
CBC	-	Complete Blood Count
CDC	-	Center for Disease Control, USA
CHIK	-	Chikungunya
CSF	-	Cerebrospinal Fluid
DDT	-	Dichloro-diphenyl Trichloroethane
DGHS	-	Directorate General of Health Services
DHF	-	Dengue Haemorrhagic Fever
DNA	-	Deoxyribonucleic Acid
EDTA	-	Ethylene Diamine Tetraacetic Acid
EIA	-	Enzyme Immunoassay
EIDs	-	Emerging Infectious Diseases
ELISA	-	Enzyme Linked Immunosorbent Assay
HAV	-	Hepatitis A Virus
HBV	-	Hepatitis B Virus
HCV	-	Hepatitis C Virus
HDV	-	Hepatitis D Virus
HEV	-	Hepatitis E Virus
HIV	-	Human Immunodeficiency Virus
ICT	-	Immunochromatographic Test
IEDCR	-	Institute of Epidemiology, Disease Control and Research
JE	-	Japaneses Encephalitis
JEV	-	JE virus
LD	-	Leishmania Donovani
MP	-	Malarial Parasite
MT	-	Medical Technologist
NiV	-	Nipah Virus
PPE	-	Personal Protective Equipment
RDT	-	Rapid Diagnostic Test
RNA	-	Ribonucleic Acid
SOP	-	Standard Operating Procedure
WHO	-	World Health Organization

Background

Biosafety, biosecurity and infection control are the essential components of all health care laboratories, research facilities, hospitals as well as veterinary hospitals and laboratories. Biosafety programs reduce or eliminate exposure of individuals, community and the environment to potentially hazardous biological agents. On the other hand, biosecurity refers to prevent loss, theft or misuse of microorganisms, biological materials, and research-related informations. Good laboratory practice ensure biosafety and infection control in the laboratory. It is impossible to achieve excellent patient care, protection of laboratory personnel and prevention of infection in the community without employing infection control procedures. 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. Though the SOP's are intended to use for public health laboratories network, recent outbreak of zoonotic diseases needs experience/ technology sharing between veterinary and public health laboratories.

Bangladesh has a well established infrastructure of health care delivery system. Laboratory service has been running from primary to tertiary level hospitals as well as specialized institutes. In addition to that veterinary laboratories are also available for animal health from primary to tertiary level. These laboratories should adopt or follow defined infection control procedures. Laboratories are practicing biosafety and infection control but it needs harmonization. To overcome these problems WHO has taken the initiatives to develop the SOP's of biosafety, biosecurity and infection control for laboratories.

Objective

The SOP's has been developed to enable health workers and laboratory personnel for safe handling of clinical specimens during collection, storage, transportation as well as safe decontamination and disposal of laboratory waste to avoid laboratory acquired infection.

Chapter I: Biological Risk Assessment

Biological Risk Assessment

The backbone of biosafety practice is risk assessment of microorganisms. The following factors are considered for biological risk assessment

- ❒ Pathogenicity
- ❒ Infectious dose
- ❒ Mode of transmission
- ❒ Host range
- ❒ Availability of effective vaccine
- ❒ Availability of effective treatment

Classification of microorganisms by risk group *(Description of Biosafety Level Laboratories- Appendix no-1)*

Table 1: Risk Group

Risk group	Definition	Example	Biosafety level
Group 1 (no or low risk for individual and community)	A microorganism that is unlikely to cause human or animal disease	*See appendix*	BSL 1
Group 2 (moderate risk for individual and low risk for community)	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.	*See appendix*	BSL 2
Group 3 (high risk for individual, low risk for community)	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.	*See appendix*	BSL 3
Group 4 (high risk for individual and community)	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.	*See appendix*	BSL 4

Laboratory Biosafety Manual, WHO, 3rd Edition

In Bangladesh BSL1 standard laboratories are available at primary and secondary health care facilities. All tertiary level hospital and specialized Institutes have BSL2 facilities. For this reason following laboratory practices are adopted.

Table 2: Type of specimen, Test methods and Biosafety level

Type of specimen	Test method	Biosafety level
Serum	Serological (ELISA, ICT)*	BSL1
Whole blood	CBC	BSL1
Sputum	Gram staining/ culture	BSL1
Urine	R/M/E, Culture	BSL1
Stool	R/M/E, Culture	BSL1
Swab	R/M/E, Culture	BSL1

NB:

a. Above test methods for risk group 2 can be performed in BSL1 with appropriate PPE where BSL 2 is not available

b. Risk group 3 should be handled according to WHO laboratory biosafety manual. But some serological tests (HIV, Dengue, Chikungunya, M. tuberculosis, Salmonella spp.) can be performed in BSL1 with appropriate PPE where BSL2 is not available

c. Risk group 4 should be handled according to WHO laboratory biosafety manual

Chapter II: Good Laboratory Practices

Good Laboratory Practices:

Good Laboratory Practice (GLP) embodies a set of principles that provides a framework within which laboratory works are planned, performed, monitored, recorded, reported and archived.

General safety measures in laboratory:

1. Wear laboratory shoes toe covered
2. Wash hands
3. Wear laboratory coat/ gown
4. Do not eat, drink or smoke
5. Do not store food, drinking water or beverage in the refrigerators
6. Do not apply cosmetics
7. Maintain nail hygiene
8. Cover hair and beard
9. Avoid work with open cuts and broken skin (if required, cover it with water seal bandage)
10. Take necessary vaccine(s) (HBV, TB, Rabies, flu, Typhoid vaccine etc)
11. Inform the laboratory in-charge in case of any emergency
12. Do not attend mobile phone during work
13. Avoid mouth pipetting

A. Safe handling of specimens in the laboratory:

Specimens submitted for laboratory testing require proper handling from the time of collection through all stages of transportation, storage, and festing. Quality laboratory results depend on all of these issues. To minimize the potential exposure hazards follow the specific SOP on collection, transportation and storage of laboratory samples in specific section.

a. Specimen-wise container selection-

For proper laboratory testing it is essential to select the appropriate container for specific test.

Table-3: Specimen types and Containers (for details see appendix - II)

Specimens	Containers
1. Whole blood	Vacutainer or test tube (with specific anticoagulant)
2. Serum	Vacutainer (without anticoagulant) or Plain test tube
3. Plasma	Vacutainer or test tube (with specific anticoagulant)
4. Stool	Disposable plastic wide mouth screw cap container
5. Urine	Disposable plastic wide mouth screw cap container
6. Sputum	Disposable plastic wide mouth sterile container
7. Body fluids	Disposable syringe or sterile test tube/vials
8. Tissue/organs for Histopathology	Disposable plastic wide mouth sterile container with 10% formalin
9. Tissue/organs for Microbiology and molecular test	Disposable plastic wide mouth sterile container with or without preservative*
10. Swabs	Sterile Swab Stick (Cotton/Dacron/Alginate- where applicable) in test tube/ Blister.

NB: *For culture, specimens container must be sterile*
For molecular test specimen container must be nuclease free

b. Preparation of the specimen containers

- Check specimen containers for leak proof and properly fit screw cap
- Label the containers with adhesive sticker (ID no, Name, Type of specimen and Date of collection)
- Use permanent marker for labeling
- Clean the outer surface of the specimen containers
- Follow standard procedure for reusable specimen containers
- For reusable containers ensure that the containers are clean, decontaminated and dry.

c. General precautions for specimen collection:

Proper specimen collection is the most important steps for any laboratory testing. To prevent contamination and to protect laboratory personnel from unexpected exposure, general precaution measures should be taken (SOP-8).

d. General Precautions for specimen transport:

Transportation of specimen from collection site to the laboratory has a great impact on quality result. To minimize cross contamination and exposure to infectious substance during transportation follow the (SOP-6).

e. General Precautions for specimen receive:

There are possibilities of accidental exposure to potentially hazardous infectious materials during sample receiving. To minimize accidental exposure follow (SOP-12).

B. Commonly used PPEs for GLP:

- Gloves- Disposable surgical gloves / Utility gloves
- Gown
- Hair cover/ cap
- Mask (surgical/ N95 particulate respirator)
- Boots/ shoe cover
- Face shield/ goggles

Procedure of wearing/removal/disposal of PPE follow the instruction from standard operating procedure section.

C. Decontamination of infectious material:

All clinical specimens must be considered as infectious materials. It is essential to decontaminate both reusable (work surface, equipments) and disposable items before reuse or final disposal.

All specimens are regarded as potentially infectious

a. Laboratory and laboratory equipments: (See the list of equipments in Appendix-III)

- Laboratory premises should keep clean and decontaminate regularly.
- Everyday before and after works, clean and decontaminate work surface with hypochlorite solution followed by 70% alcohol.
- Clean and decontaminate equipments like pipette, bio-safety cabinet, centrifuge machine, vortex mixture and others laboratory equipments with 70% alcohol or 0.5% hypochlorite solution
- Clean laboratory floor with phenol or lyzol regularly or 0.5% hypochlorite solution

b. Laboratory disposable items:

- Decontaminate all disposable items before disposal, preferably with autoclave
- In case of unavailability of autoclave, clean all items with detergent and running water, and then submerge in hypochlorite solution for at least 30-40 minutes.

c. Laboratory reusable items:

- Remove all visible organic debris by washing in detergent solution.
- Decontaminate the reusable items with autoclave / hot air oven / hypochlorite solution / 70% alcohol as appropriate.

D. Waste management in the laboratory:

Infectious waste contains a great variety of pathogenic microorganisms. Pathogens in infectious waste may infect human through puncture, abrasion or cut in the skin; through the mucous membrane; by inhalation or accidental ingestion.

Waste management includes:

- Collection and segregation of waste
- Processing (decontamination) of waste
- Transportation of waste for disposal
- Table-3 shows the management of laboratory waste in short.

For standard procedure of managing waste follow the standard operating procedure from specific section.

Table-4: Laboratory waste management according to category

Waste category	Description and Examples	Management
Infectious/ contaminated waste	**Non degradable** **1. Sharp** Hypodermic needles, scalpels, broken lab glassware & Plastic ware, blades	**Segregation-** - Puncture-proof colour coded container - If colour coded container not available then use any beaker/ bucket containing 0.5% hypochlorite solution **Processing before disposal** Sterilize by Autoclave/ decontaminate 0.5% hypochlorite solution **Transport to the place of disposal** Cleanable trolley **Disposal** Incineration / Land fill
	2. Non Sharp Syringes,infusion sets, ELISA plate, ICT, micro centrifuge tube, petri dish, Vacutainer, plastic tube, Pipette tips, mask, gloves	**Segregation-** - Colour coded container - If colour coded container not available then use any beaker/ bucket containing 0.5% hypochlorite solution **Processing before disposal** Sterilize by Autoclave/ decontaminate by 0.5% hypochlorite solution **Transport to the place of disposal** Cleanable trolley **Disposal** Incineration / Land fill

Waste category	Description and Examples	Management
	Degradable Blood , Serum, Plasma, Urine, Stool, Swab, Body fluids, Tissues and organs	**Segregation-** - Colour coded container - If colour coded container not available then use any beaker/ bucket containing 0.5% hypochlorite solution **Processing before disposal** Sterilize by Autoclave/ decontaminate by 0.5% hypochlorite solution **Transport to the place of disposal** Cleanable trolley **Disposal** Incineration / Land fill
Non infectious household waste	Tissue paper, paper etc.	**Segregation-** - Colour coded container Processing before disposal None **Transport to the place of disposal** Cleanable trolley **Disposal** Incineration / Land fill/ Municipal disposal
Chemical waste	Non-hazardous	Non-hazardous waste- directly dispose into trash
	Hazardous if it has at least one of the following properties : • corrosive (acids and bases): hydrochloric acid , sodium hydroxide	• keep in a separate, sealable container with labeling of chemical waste • dilute strong acid and base into neutral pH and disperse into sewerage system**

Waste category	Description and Examples	Management
	flammable: alcohol, ether, acetone, acetic acid • oxidizer: conc. Hydrogen peroxide, potassium permanganate, bleach • Air or water reactive: zinc dust, magnesium metal • toxic (poisons, carcinogens, mutagens): mercury, formaldehyde, ethidium bromide	• dilute formalin in water and disperse into sewerage system • ethidium bromide in electrophoresis gel- <1%- lab trash >1%- keep in biohazard bag and incinerate • ethidium bromide in aqueous solution- <10 µg/ml- release into drain >10 µg/ml- charcoal filtration for deactivation
Wastes with high content of heavy metal	broken thermometers; blood-pressure gauges	buried in deep landfill
Radioactive waste	Unused substances from radiotherapy or laboratory research, contaminated glassware, packages or absorbent paper; urine or excreta from patients treated or tested with unsealed radioactive substance	Follow individual laboratory's own system for disposal of radioactive waste

**Sharps disposal containers must not be discarded in landfills*

** add acids/ bases in water, DO NOT ADD WATER IN ACIDS/BASES

E. Laboratory emergency management

Types of laboratory emergencies

- ❒ Spillage of clinical specimen and chemical
- ❒ Contact exposure
- ❒ Needle stick injuries
- ❒ Accidental inhalation or ingestion
- ❒ Fire/ Explosion
- ❒ Natural disaster (earthquake, storm, cyclone)

Every emergency in laboratory should manage meticulously following the standard operating procedure described in specific section of this book.

Chapter III: Biosecurity

Biosecurity

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

Objective of biosecuruty

To prevent loss, theft or misuse of microorganisms, biological materials, and research-related information.

Elements of a Biosecurity Program

Biosecurity program components should be site-specific and based upon organizational threat/vulnerability assessment and as determined appropriate by facility management. Elements discussed below should be implemented, as needed, based upon the risk assessment process. They should not be construed as "minimum requirements" or "minimum standards" for a biosecurity program.

Program Management

If a biosecurity plan is implemented, institutional management must support the biosecurity program. Appropriate authority must be delegated for implementation and the necessary resources provided to assure program goals are being met. The biosecurity program should be integrated into relevant institutional policies and plans.

Physical Security—Access Control and Monitoring

The physical security elements of a laboratory biosecurity program are intended to prevent the removal of assets for non-official purposes. An evaluation of the physical security measures should include a thorough review of the building and premises, the laboratories, and biological material storage areas.

Access should be limited to authorized and designated employees based on the need to enter sensitive areas. Methods for limiting access could be as simple as locking doors or having a card key system in place. The need for entry by visitors, laboratory workers, management officials, students, cleaning/maintenance staff, and emergency response personnel should be considered.

Personnel Management

Personnel management includes identifying the roles and responsibilities for employees who handle, use, store and transport dangerous pathogens and/or other important assets. Policies should be developed for personnel and visitor identification, visitor management, access procedures, and reporting of security incidents.

Inventory and Accountability

Material accountability procedures should be established to track the inventory, storage, use, transfer and destruction of dangerous biological materials and assets when no longer needed. The objective is to know what agents exist at a facility, where they are located, and who is responsible for them. To achieve this, management should define: 1) the materials (or forms of materials) subject to accountability measures; 2) records to be maintained, update intervals and timelines for record maintenance; 3) operating procedures associated with inventory maintenance (e.g., how material is identified, where it can be used and stored); and 4) documentation and reporting requirements.

It is important to emphasize that microbiological agents are capable of replication and are often expanded to accommodate the nature of the work involving their use. Therefore, knowing the exact "working" quantity of organisms at any given time may be impractical. Depending on the risks associated with a pathogen or toxin, management can designate an individual who is accountable, knowledgeable about the materials in use, and responsible for security of the materials under his or her control.

Information Security

Policies should be established for handling sensitive information associated with the biosecurity program. For the purpose of these policies, "sensitive information" is that which is related to the security of pathogens and toxins, or other critical infrastructure information. Examples of sensitive information may include facility security plans, access control codes, agent inventories and storage locations.

The objective of an information security program is to protect information from unauthorized release and ensure that the appropriate level of confidentiality is preserved. Facilities should develop policies that govern the identification, marking and handling of sensitive information. Policies for properly identifying and securing sensitive information including electronic files and removable electronic media (e.g., CDs, computer drives) should be developed.

Transport of Biological Agents

Material transport policies should include accountability measures for the movement of materials within an institution (e.g., between laboratories, during shipping and receiving activities) and outside of the facility (e.g., between institutions or locations). Transport policies should address the need for appropriate documentation and material accountability and control procedures for pathogens in transit between locations. Transport security measures should be instituted to ensure that appropriate authorizations have been received and that adequate communication between facilities has occurred before, during, and after transport of pathogens or other potentially hazardous biological materials. Personnel should be adequately trained and familiar with regulatory and institutional procedures for proper containment, packaging, labeling, documentation and transport of biological materials.

Accident, Injury and Incident Response Plans

Laboratory security policies should consider situations that may require emergency responders or public safety personnel to enter the facility in response to an accident, injury or other safety issue or security threat. The preservation of human life, the safety and health of laboratory employees and the surrounding community must take precedence in an emergency over biosecurity concerns. Facilities are encouraged to coordinate with medical, fire, police and other emergency officials when preparing emergency and security breach response.

Reporting and Communication

Communication is an important aspect of a biosecurity program. A "chain-of-notification" should be established in advance of an actual event. This communication chain should include laboratory and program officials, institution management, and any relevant regulatory or public authorities. The roles and responsibilities of all involved officials and programs should be clearly defined. Policies should address the reporting and investigation of potential security breaches (e.g., missing biological agents, unusual or threatening phone calls, unauthorized personnel in restricted arcas).

Training and Practice Drills/ reharsal

Biosecurity training is essential for the successful implementation of a biosecurity program. Program management should establish training programs that inform and educate individuals regarding their responsibilities within the laboratory and the institution. Practice drills should address a variety of scenarios such as loss or theft of materials, emergency response to accidents and injuries, incident reporting and identification of and response to security breaches. These scenarios may be incorporated into existing emergency response drills such as fire drills or building evacuation drills associated with bomb threats. Incorporating biosecurity measures into existing procedures and response plans often provides efficient use of resources, saves time and can minimize confusion during emergencies.

Security Updates and Re-evaluations

The biosecurity risk assessment and program should be reviewed and updated routinely and following any biosecurity-related incident. Reevaluation is a necessary and on-going process in the dynamic environments of today's biomedical and research laboratories. Biosecurity program managers should develop and conduct biosecurity program audits and implement corrective actions as needed. Audit results and corrective actions should be documented. The appropriate program officials should maintain records.

Chapter IV: Standard Operating Procedures (SOPs)

Standard Operating Procedure (SOP) for Laboratory House Keeping

SOP no and version	**01**
SOP name	Standard Operating Procedure for Laboratory house keeping

Purpose:

- To ensure a clean, safe and pleasant work environment
- To reduce risks and protects the integrity of biological experiments.
- To provide work areas free of significant sources of contamination.
- To protect laboratory personnel from exposure of unwanted incidents.

Logistics required:

A. Equipment

B. Chemical/reagents

- Disinfectant
- Antiseptic floor cleaner
- Insecticides
- Temperature log sheet
- Mop
- PPEs

Responsibilities:

- Laboratory incharge

General instructions:

1. Work Environment

A. Put hazard warning symbols in the door. (For different symbols see the appendix-IV)
B. Ensure adequate ventilation and lighting in the work area
C. Ensure regular cleaning of laboratory floor maintaining a log book
D. Make Corridors and spaces free of hazardous materials
E. Deal all spills immediately
F. Clean bench tops every day before and after work
G. Have a designated room for storage
H. Place containers and equipment away from the edge of benches to avoid them from being knocked over
I. Keep shelves above the work bench orderly: heavy item on the lower shelves, lighter on the upper shelves, do not overload the shelves as falling items may injure laboratory workers and ruin experiments
J. Cover chairs with non-fabric material
K. Replenish Soap and paper towels near hand-wash basins regularly
L. Hang up the lab coats (Apron) inside the lab
M. Keep personal belongings in lockers outside the lab
N. Pay attention to electrical safety
O. Keep the designated bins for biohazard waste and sharps in convenient location
P. Dispose wastes safely and promptly
Q. Rectify unsafe conditions as soon as possible

2. Chemicals

A. Store bulk chemicals in their designated cabinets or store-rooms and not on the bench top or lab floor
B. Keep working chemicals at proper place with proper label
C. Cap and seal all containers tightly to reduce the possibility of a spill and release of fumes into the lab except when being used

D. Keep flammable items away from hot and fire sources.
E. Strap all compressed gas cylinders securely to prevent them from falling over
F. Dispose all unused or expired chemicals promptly and safely according to recommended guidelines
G. Maintain a good up to date chemical inventory for ease of tracking

3. Emergency Equipment

Keep the following emergency equipment in view and accessible.

A. Telephone
B. Wash sinks
C. First aid kit
D. Fire extinguisher
E. Fire alarm
F. Eye wash station / emergency shower
G. Spill kits
H. MSDS

Emergency equipment must be properly maintained and regularly serviced

Standard Operating Procedure (SOP) for Hand Washing

SOP no and version	02
SOP name	Standard Operating Procedure for hand washing

Purpose: Infection control

Logistics/ Materials required: running water and soap/ alcohol based hand rub

Users: All laboratory personnel

When to do:

1. Arrival at lab work place
2. Before leaving lab work place
3. Before and after any laboratory work

Procedure:

With soap:

1. Wet hands with clean running water
2. Scrub fingers, palms, backs of hands, wrist and forearms (follow diagram) creating friction for at least 20 seconds with soap and make enough foam. Clean under nails
3. Thoroughly rinse hands under running water
4. Use paper towels to blot and dry hands

With hand sanitizer:

Apply 5 cc or 1 pump from dispenser of sanitizer into palm of hands, rub According the diagram above.

Remember

a. Remove all jewelries (rings, bracelets) and watches
b. Ensure that the nails are clipped short
c. Do not wear artificial nails
d. Roll the sleeves up to the elbow

Standard Operating Procedure for Wearing, Removal and Disposal of PPE

SOP no and version	**03**
SOP name	Standard Operating Procedure for Wearing, Removal and disposal of PPE

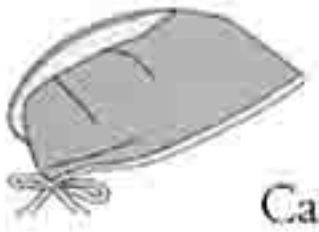
Purpose: Biosafety and infection control

Logistics/ Materials required:

Gown

Boots/ Shoe cover

Mask/ N95 particulate respirator



Cap

Face shield

Goggles

Gloves

1. Gown
2. Boots
3. shoe cover
4. Mask
5. N95 particulate respirator
6. Laboratory cap
7. Lab goggles
8. Gloves

Responsibilities:

- Self responsibility

Steps of wearing of PPE:

1. Gather all the necessary items of the PPE beforehand.
2. Put on the scrub suit in the changing room.
3. Put on gumboots; If not available, make sure you have closed, puncture and fluid resistant shoes and put on shoe cover.

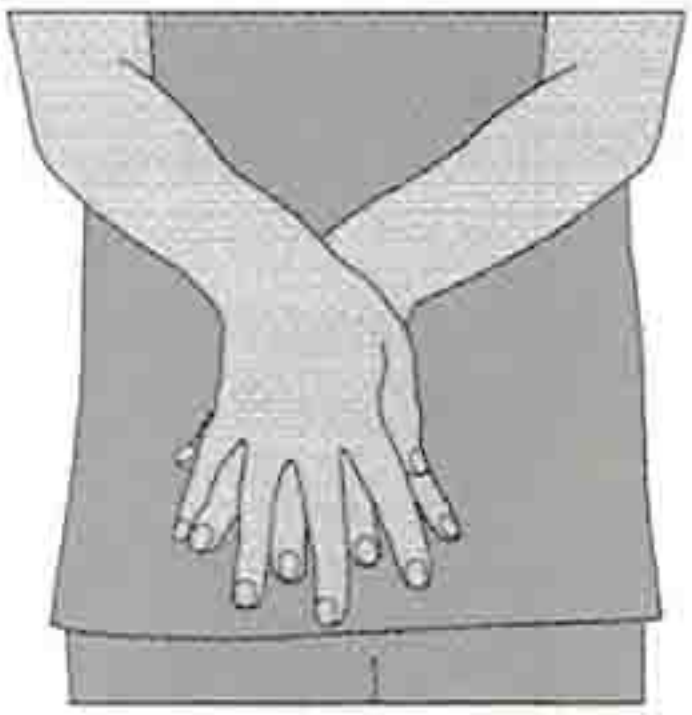
4. Place the gown over the scrubs. If a impermeable gown is not available, place waterproof apron over gown.

5. Put on mask (either surgical mask or N95 particulate respirator)

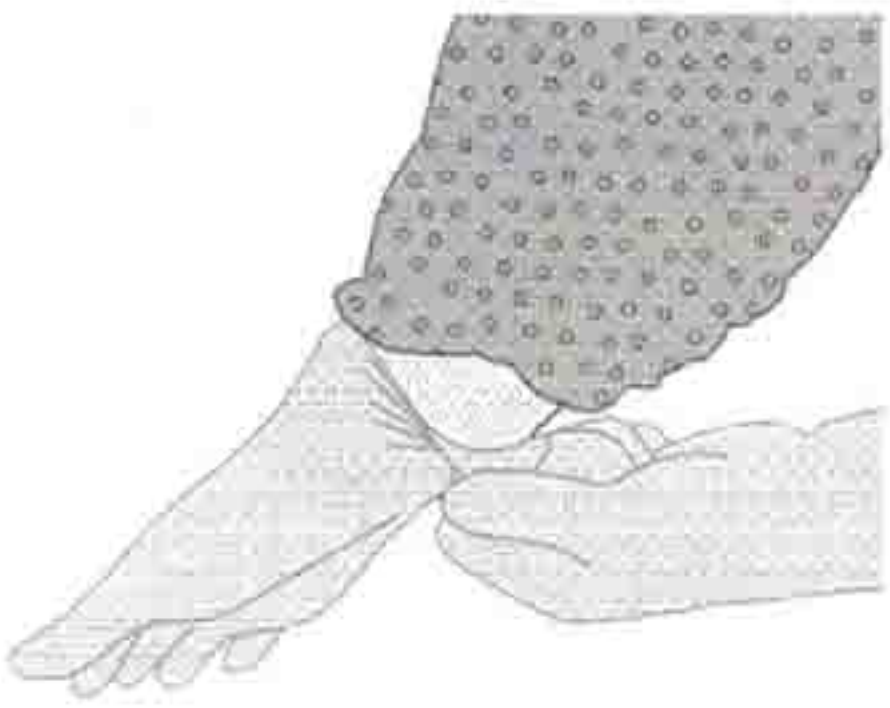
6. Put on goggles or a face shield

7. Put on hair cover/ cap

8. Use hand rub



9. Put on gloves



Remember

a) Avoid touching or adjusting PPE
b) Remove gloves if they become torn or damaged
c) Change gloves between patients
d) Perform hand hygiene before donning new gloves

Steps of removal and disposal of PPE:

1. Peel off Gown/apron.

- If reusable decontaminate with detergent water/0.05% hypochlorite solution for 30 minutes then send for autoclaving
- If disposable, dispose off in biohazard bag/ color coded bin.

2. Remove shoe cover with your gloves still on and dispose off shoe cover in biohazard bag/ color coded bin

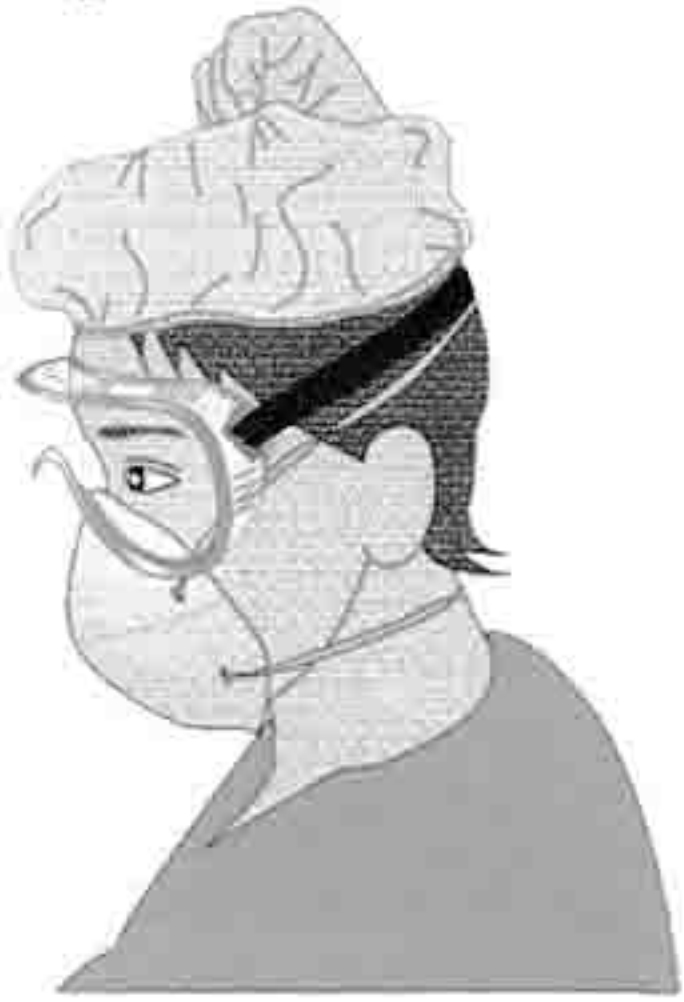
- If wearing gum boots, remove them (ideally using the boot remover) without touching them with your hands.
- Place the removed boots into a container with disinfectant.

3. Remove gown and gloves and roll inside-out.

- Dispose of gloves and disposable gown in biohazard bag/ color coded bin.
- If reusable decontaminate with detergent water/0.05% hypochlorite solution for 30 minutes then send for autoclaving

4. Remove cap/ hair cover (from behind head).

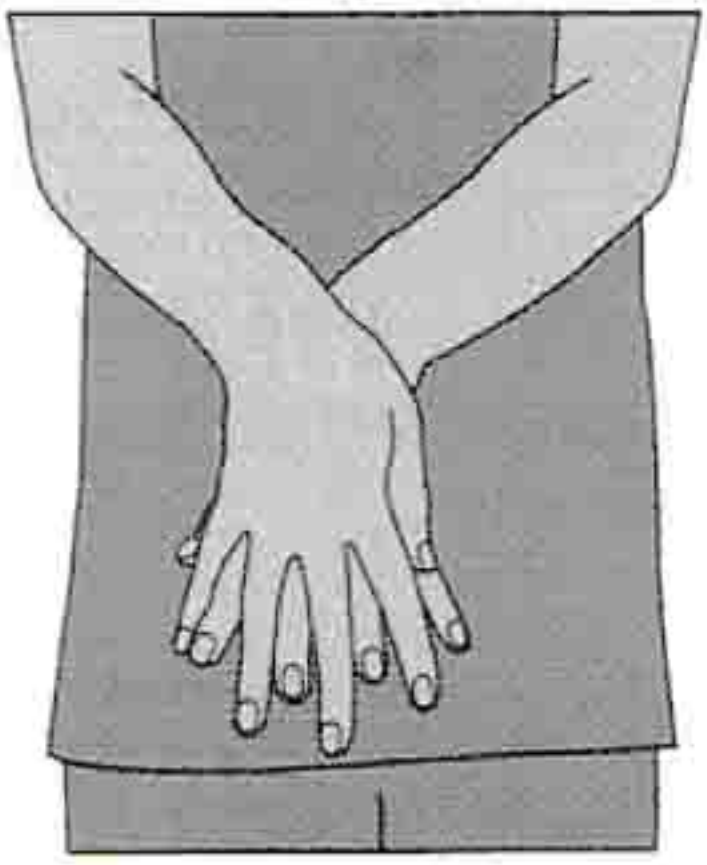
- If reusable decontaminate with detergent water/0.05% hypochlorite solution for 30 minutes then send for autoclaving
- If disposable, dispose off in biohazard bag/ color coded bin



5. **Remove goggles (from behind head). Disinfect it with 70% alcohol.**

6. **Remove mask from behind head and dispose of mask in biohazard bag/ color coded bin.**

7. **Perform hand hygiene.**



Standard Operating Procedure (SOP) for Wearing of Respirator and Seal Checking Procedure

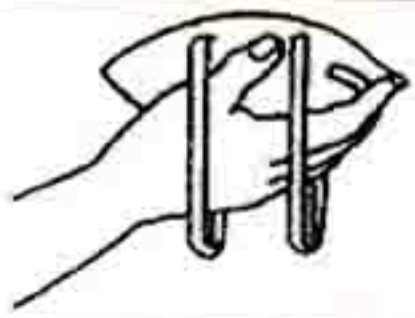
SOP no and version	**04**
SOP name	Standard Operating Procedure for Wearing of Respirator and Seal checking procedure

Logistics/ Materials required: N95 particulate respirator

Users: All laboratory personnel

Procedure:

Wearing of the Respirator & seal-checking procedures

1) Hold the respirator in one hand, with the nose piece at the fingertips and let the head straps hang loosely in front of the respirator	
2) Place respirator under the chin, with the nosepiece up. While holding the respirator with one hand, pull the top strap over your head, resting it at the top back of your head. Pull the bottom strap over your head, and place it around your neck, below your ears.	
3) Using both hands, mold the nose piece to the shape of your nose by pushing inward with your fingertips. Note that pinching the molding piece with one hand will likely result in less effective respirator fit.	
4) Seal-check: cover respirator completely w/ both hands, and exhale sharply. If air blows on your face or eyes, readjust the respirator according to Steps 3 & 4. Do not use respirators until you pass the seal-check (no leakage).	
5) To remove the respirator, place the hand inside the respirator. With the other hand, pull the bottom strap over your head, and then pull the top strap off. *If respirator was used in a medical facility or if there is any evidence that respirator may be contaminated, dispose of it as a bio-hazardous waste*	

Standard Operating Procedure (SOP) for Wearing and Removal of gloves

SOP no and version	**05**
SOP name	Standard Operating Procedure for Wearing and removal of gloves

Logistics/ Materials required: Gloves

Users: All laboratory personnel

Procedure:

Steps of wearing and removal of gloves:

When the hand hygiene indication occurs before a contact requiring glove use, perform hand hygiene by rubbing with an alcohol-based handrub or by washing with soap and water.

I. HOW TO DON GLOVES:

1. Take out a glove from its onginal box

2. Touch only a restricted surface of the glove corresponding to the wrist (at the top edge of the cuff)

3. Don the first glove

4. Take the second glove with the bare hand and touch only a restricted surface of glove corresponding to the wrist

5. To avoid touching the skin of the forearm with the gloved hand, turn the external surface of the glove to be donned on the folded fingers of the gloved hand, thus permitting to glove the second hand

6. Once gloved, hands should not touch anything else that is not defined by indications and conditions for glove use

II. HOW TO REMOVE GLOVES:

1. Pinch one glove at the wrist level to remove it, without touching the skin of the forearm, and peel away from the hand, thus allowing the glove to turn inside out

2. Hold the removed glove in the gloved hand and slide the fingers of the ungloved hand inside between the glove and the wrist. Remove the second glove by rolling it down the hand and fold into the first glove

3. Discard the removed gloves

4. Then, perform hand hygiene by rubbing with an alcohol-based handrub or by washing with soap and water

Standard Operating Procedure (SOP) for Transport of Specimens

SOP no and version	**06**
SOP name	Standard Operating Procedure for transport of specimens

Logistics/ Materials required:

1. PPE
2. Primary, secondary and tertiary containers
3. Cool box with ice pack

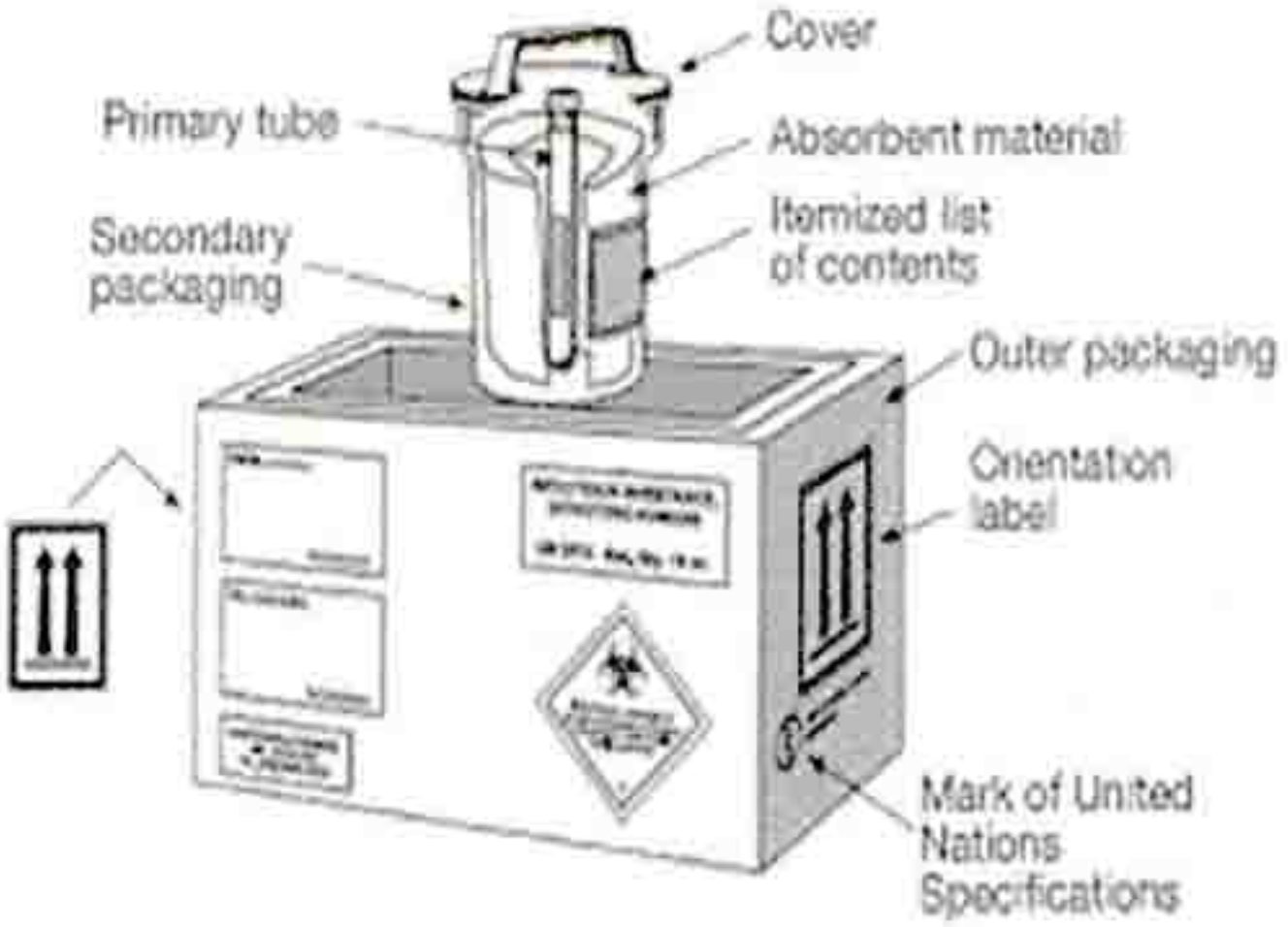
Responsibilities:

1. Laboratory personnel in Charge
2. Lab attendant

Procedures:

1. Wash hands
2. Open the secondary container before handling the primary container
3. Wear gloves
4. Use leak proof screw cap as primary container
5. Use absorbent and shock proof materials around primary container
6. Place Primary container within a secondary leak proof container
7. Remove gloves and dispose in biohazard bag
8. Place list of specimens and laboratory advice form outside of secondary container
9. Place the secondary container within an outer package with appropriate labeling
10. Use secondary containers* (such as boxes) fitted with racks so that the specimen containers remain upright to avoid accidental leakage or spillage
11. Autoclave or decontaminate (with chemical disinfectant) the secondary container regularly

Transport and storage of specimen vary according to types of sample and organism to be isolated



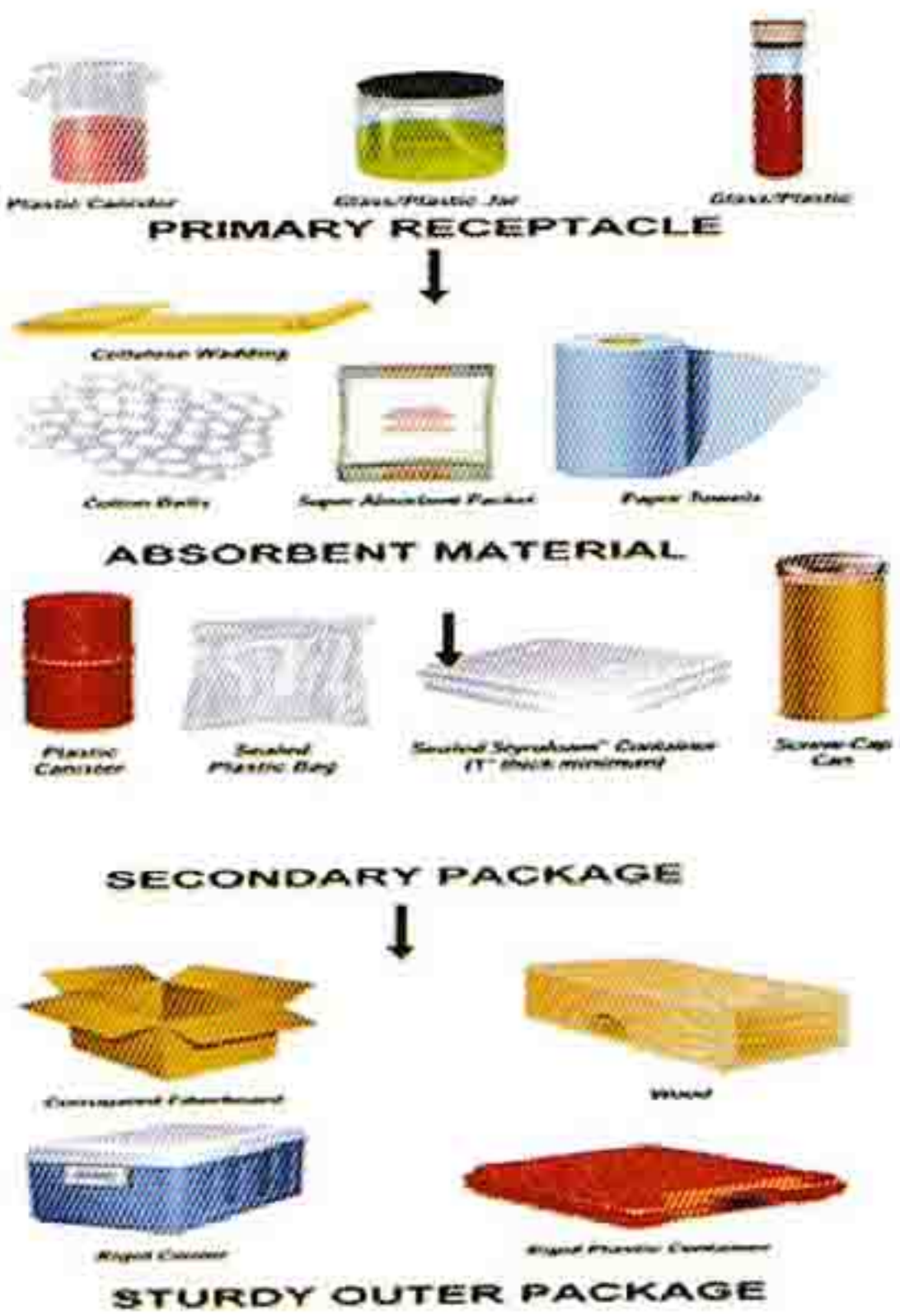


Fig: Triple Packaging

** The secondary container is made of metal or plastic with a gasket seal, autoclavable or resistant to the action of chemical disinfectants*

Standard Operating Procedure (SOP) for Storage of Specimens

SOP no and version	07
SOP name	Standard Operating Procedure for storage of specimens

Purpose: Biosafety and infection control

Logistics/ Materials required:

1. PPE
2. Freezer (<-20^0 C)
3. Refrigerator (2-8^0 C)
4. Ultra low temperature freezer (<-70^0 C)
5. Liquid nitrogen tank (-196^0 C)
6. Sample box
7. Temperature log sheet
8. Register book

Responsibility:
Laboratory in Charge

Procedures:

1. Wear PPE
2. Place the specimen in box with proper labeling
3. Keep the box in refrigerator/ freezer
4. Do not keep different types of sample in the same box
5. Keep the box with different types of sample in different site of refrigerator/ Freezer
6. Keep reagents and sample in separate refrigerator/ Freezer
7. Maintain a temperature log sheet regularly
8. Maintain register book.

Standard Operating Procedure (SOP) for Infection Control During Specimen Collection

SOP no and version	**08**
SOP name	Standard Operating Procedure for infection control during Specimen collection

Purpose:

1. To minimize the risk of exposure during specimen collection
2. To reduces risks and protects the integrity of biological sample

Logistics required:

1. Designated container
2. Marker
3. Bar code sticker
4. Rack
5. Tourniquet
6. Disposable syringe, Swab stick
7. Disposable tongue depressor
8. Discard bin (Colour coded)
9. Disinfectant
10. Cool box
11. PPEs
12. Designated room/ corner

Responsibilities:

1. Laboratory incharge
2. Biosafety officer
3. Phlebotomist
4. Lab attendent
5. Cleaner

General precautions for specimen collection:

1. History taking relevant to disease and organism
2. Instruct properly for patient preparation
3. Check for the complete request form and confirm the identity of the patient

4. Check for the specimen container is labeled correctly
5. Check that the patient is appropriately prepared (eg- fasting, morning urine etc)
6. Ensure that the specimen is collected properly
7. Ensure that environmental and storage conditions are fulfilled to protect specimens from deterioration
8. Ensure the safe disposal of all materials used in specimen collection
9. Ensure that high risk specimens are identified and processed correctly
10. Ensure that all spillages and breakages are dealt with correctly.
11. Maintain following precaution to minimize risk of exposure-

If no sharps container: collect sharps immediately to prevent needle-stick injury

Have first aid kit readily accessible

Do not reuse contaminated equipment

Precautions	Use	Requirements
Contact precautions	Patients known or suspected to have serious illnesses easily transmitted by direct patient contact or by contact with items in the patient's environment	• Gloves • Gown
Droplet precautions	Barrier to stop infections spread by large (>5 microns), moist droplets produced by people when they cough, sneeze or speak	• Contact precautions • Well-fitting mask • Eye protection
Airborne precautions	Patients known or suspected to have serious illnesses transmitted by airborne droplet nuclei	• Contact precautions • Droplet precautions • N95 mask • Isolation room (In hospital)

Standard Operating Procedure (SOP) for Waste Segregation and Disposal

SOP no and version	**09**
SOP name	Standard Operating Procedure for waste segregation and disposal

Objectives and scope

This SOP describes the procedures for proper disposal of non-infectious waste and safe disposal of infectious and sharps waste.

Responsibility

This SOP is applicable to all employees of the laboratory.

Tasks, responsibilities and accountabilities

Task	Responsible	Accountable
Removal of non-infectious waste from lab	Support staff	Lab manager
Autoclaving infectious waste	Lab personnel working in lab	Lab manager

Procedure

A. Non-infectious waste disposal

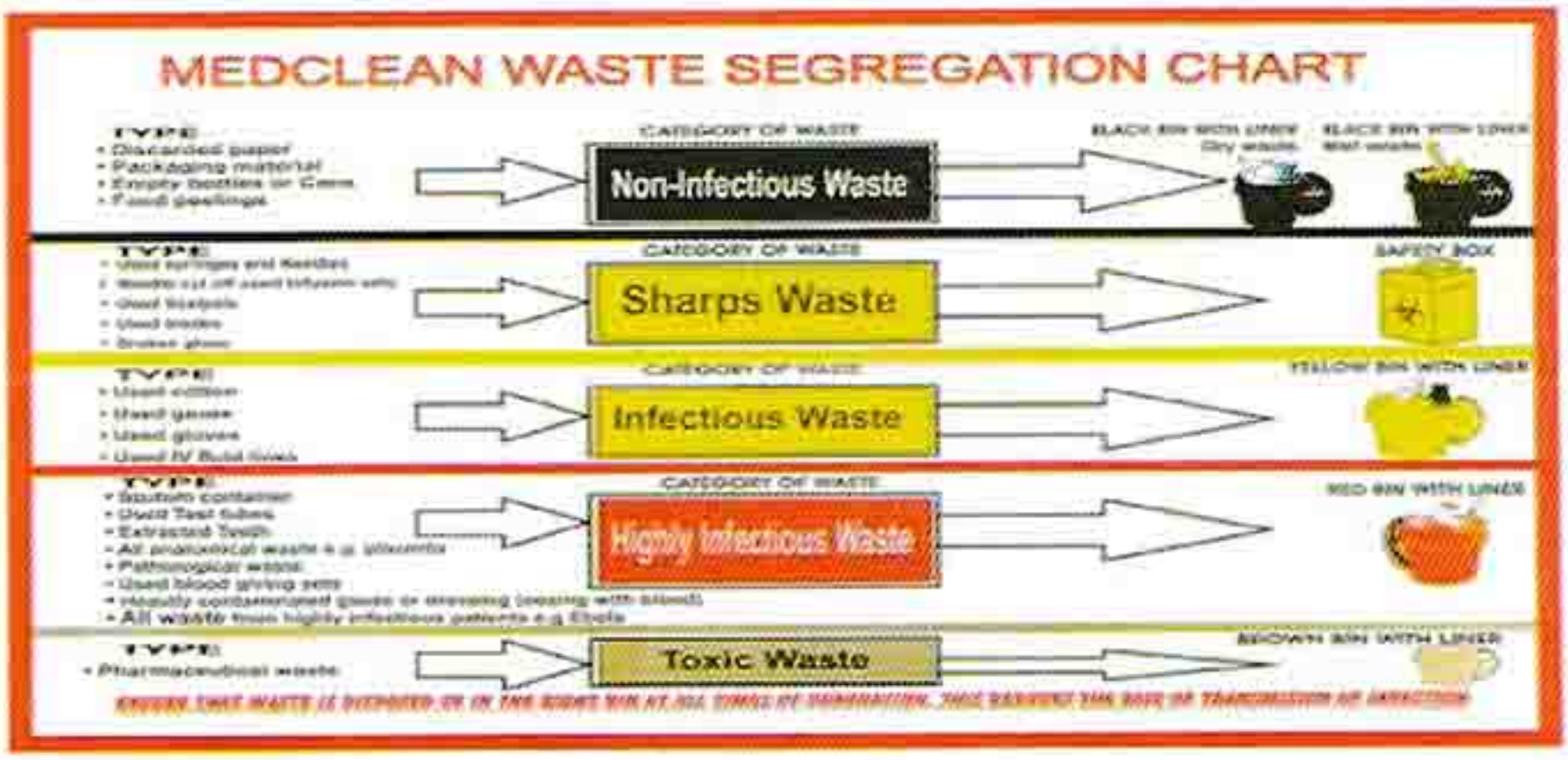
1. These include papers, packaging boxes, plastic bags and hand paper towels.
2. Dispose these items in black or white polythene bags inserted in 10L plastic buckets without a lid.
3. Remove filled bags and replace them with new ones ready for the following day work.

B. Infectious waste disposal

1. Do not store infectious waste in corridors or stairwells
2. Seal used tubes, plastic Pasteur pipettes, pipette tips, gloves, cotton, paper towels, specimen containers and cultures for discard in double biohazard bags
3. Place all infectious waste in designated red, rigid bags placed in waste bins that are clearly labeled with the biohazard symbol
4. Seal off with tape, when the bags are three-quarter full
5. Carefully take the sealed bags to the autoclave room and autoclave perform
6. After autoclaving, bio hazard bags containing reusable lab ware are safe to open and clean and take non-reusable waste for incineration

C. Disposal of sharps

1. Sharps include used needles, surgical knives or broken bottles
2. Do not mix sharps with papers or non-sharps infectious waste
3. Put a properly labeled sharps container in place and used exclusively for the disposal of sharps
4. Discard used needles immediately into the sharps container
5. Pick broken glass scattered on the floor with aid of special forceps or swept into a special receptacle and then emptied into the sharps container
6. Autoclave sharps containers when ¾-full and then sent for incineration



Liquid medium and solutions

1. Add to a container of bleach solution
2. Leave for approximately 20 minutes
3. Pour disinfected liquids down the drain
4. If using any disinfectant other than bleach, which may not be appropriate to pour down the drain, contact Environmental Safety Department for safe disposal options

Contaminated PPE

1. All disposable, contaminated PPE is disposed in a biohazard waste container
2. Autoclave before final disposal
3. Decontaminate by submerging the reusable items in 0.5% hypochlorite solution for at least 30 minutes (preferable over night), then autoclave before use

Standard Operating Procedure (SOP) for Emergency Management in Laboratory

SOP no and version	**10**
SOP name	Standard Operating Procedure for emergency management in laboratory

Purpose:

1. A standardized procedure to minimize the incidence and risk of exposure during any emergency
2. Manage the emergency situation in lab if arise

Preparedness for an emergency:

1. Keep an adequately equipped emergency response supplies i.e Spill Kit(Appendix V), First Aid Kit (Appendix VI) and Antidote, including Safety Shower/Eyewash and fire extinguisher in common place of the laboratory that is known and easily accessible to all staffs related to laboratory works
2. Certify fire extinguisher annually
3. Maintain a department emergency phone directory
4. Know the building's floor plan & emergency exit routes
5. Staff training on:
   - ❒ Use of First Aid Kit, Antidote and Spill Kit
   - ❒ Use of Safety Shower and Eyewash
   - ❒ Arrange fire training program
   - ❒ Arrangement of fire drill annually
   - ❒ Management of spills
   - ❒ Emergency exit safely
   - ❒ Medical emergencies

Develop a response plan & know how to report incidents and to whom and share preparedness information with all staff three monthly.
Notify institutional focal person incase of any incident

- Components of first aid kit:
  - Sterile dressing to cover wounds
  - Absorbent cotton wool
  - Triangular and roll bandage
  - Band aid
  - Sterile eye pad
  - Roll of adhesive tape
  - Alcohol pad
  - Safety pins
  - Scissors
  - Sodium bicarbonate powder
  - Boric acid powder
  - Pain killer
  - Proton pump inhibitor (omeprazol)
  - Antacid
  - Antiseptic lotion/cream
  - Silcream
  - blood pressure machine

Fig: First aid kit

Incident Specific Emergency Management

- Components of laboratory spill kit:
  - Disposable nitrile rubber gloves - a few pairs
  - Caution Chemical Hazard banners tape, 6' rolls - 2 each
  - Plastic bags (For disposal) - 6 each
  - UCSD Hazardous Waste Tags - 1 pad
  - Ball point pen - 1 each
  - Lab Marker Pen - 1 each
  - Container of Sodium Bicarbonate, marked "Acid Neutralizer - Sodium Bicarbonate" - 1 each
  - Container of Citric Acid, marked "Base Neutralizer - Citric Acid" - 1 each
  - Mercury Sponge containers - 1 each
  - Mercury In-Line Vacuum Trap Kit (bagged with tubing and disposal bags) - 1 each
  - pH paper - 2 vials

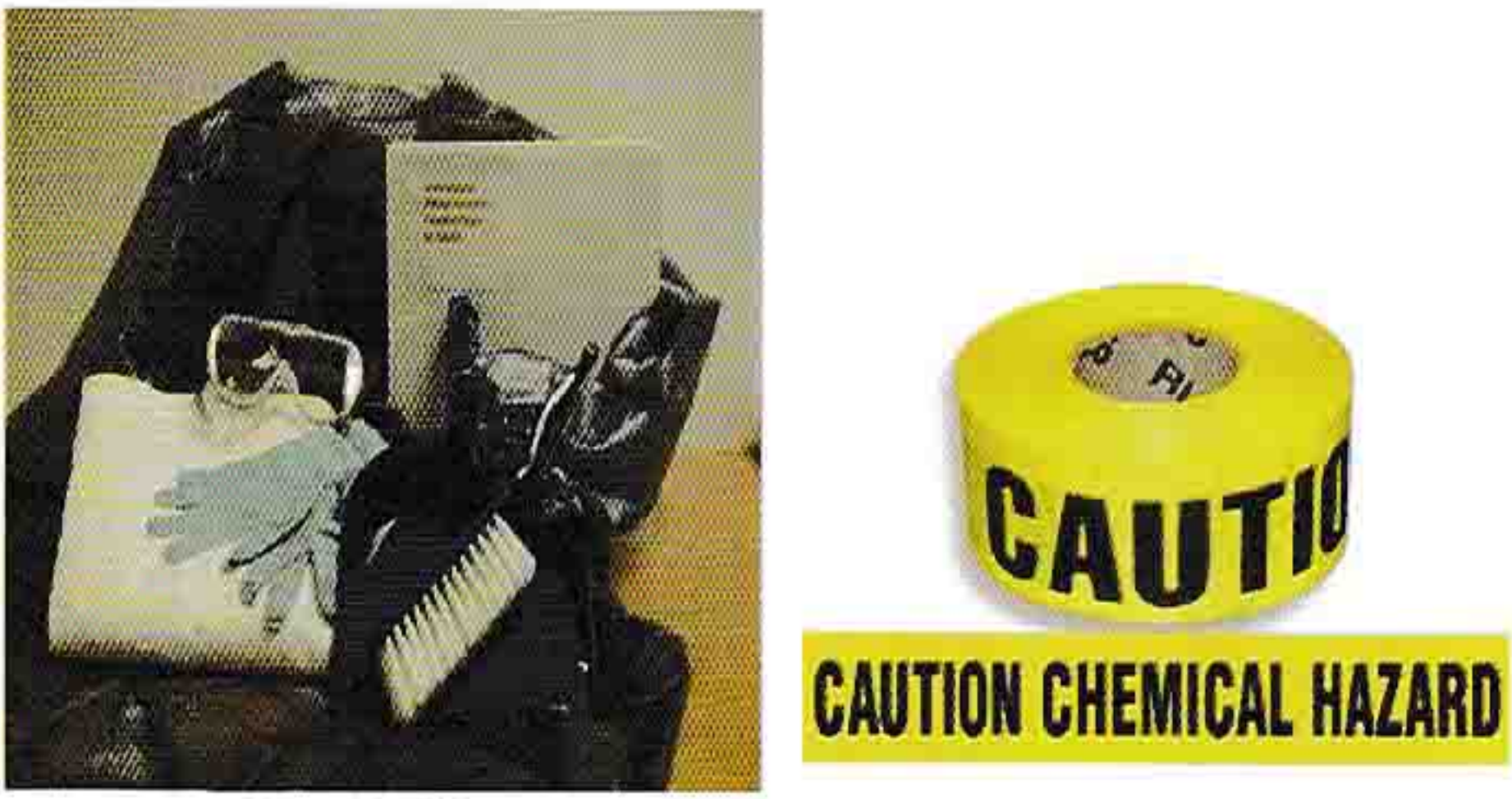


Fig: Spill kit

- **"Dry" spills (overturned or broken culture plate) with no significant aerosol formation-**
  - Put on necessary PPE such as gloves, lab coat, face shield or goggles

- ❊ Flood area with 10% bleach, soak up disinfectant and contaminated material with an absorbent material (sand, paper towels), place in a Biohazard container, and seal the container
- ❊ Wash the spill area thoroughly with disinfectant after the contaminated material has been removed
- ❊ Place the biohazard bag into another bag for transportation to the disposal site

- **"Liquid" spills (Clinical specimen and chemicals)**
  - ❊ Put on necessary PPE such as gloves, lab coat, face shield or goggles
  - ❊ Evacuate the area and do not enter for at least one hour due to aerosol formation
  - ❊ Cover the spill with an absorbent material (sand, paper towels); when absorption is complete, place in a Biohazard container, and seal the container
  - ❊ Wash the spill area thoroughly with disinfectant after the contaminated material has been removed
  - ❊ Place the biohazard bag into another bag for transportation to the disposal site

- **Spills in closed areas (centrifuge machine, incubator etc)**
  - ❊ Put on necessary PPE such as gloves, lab coat, face shield or goggles
  - ❊ Shut off the instrument
  - ❊ Do not open the machine for at least one hour
  - ❊ Use absorbent materials (sand, paper towels)
  - ❊ After removal of contaminated material, clean the instrument thoroughly with disinfectant before resuming work

- **Exposure to chemical spillage:**
  - ❊ If a chemical spill occurs that involves parts of the body, go under the safety shower & wash off the chemicals before severe skin burns

- **Contact exposure:**
  - * Remove lab coat, gloves and place in a biohazard bag
  - * If contaminated skin or open wounds, wash affected area vigorously with mild soap and water
  - * If contact with mucous membrane, wash with copious amounts of water, flush eyes for at least 15 min.
  - * Use safety shower if needed

Fig: Safety shower

Fig: Eye wash station

Fig: Eye wash bottle

- **Accidental needle stick injury:**
  - ❊ Immediately wash the site well with soap and water
  - ❊ Consult with physician through institutional focal person

- **Accidental inhalation and ingestion:**
  - ❊ Seek medical help immediately

- **Fire explosion:**
  - ❊ Remain calm and activate the fire alarm or call the emergency response number
  - ❊ Use fire extinguisher (Appendix VII)
  - ❊ Report to your assembly point/area by the safest route available
  - ❊ Assist others without endangering your safety
  - ❊ Walk, do not run
  - ❊ Evacuate the building. Do not waste time turning off equipment, collecting papers, or gathering personal property. Feel the doors to see if they are hot before opening them. If there is smoke present, crawl low because the air is fresher and cooler
  - ❊ Use stairs, do not use elevators
  - ❊ Do not enter the building after evacuation without fire department approval

Note: It's easy to remember how to use a fire extinguisher if you can remember the acronym PASS, which stands for Pull, Aim, Squeeze, and Sweep.

	**Pull the pin.** This will allow you to discharge the extinguisher.
	**Aim at the base of the fire.** If you aim at the flames (which is frequently the temptation), the extinguishing agent will fly right through and do no good. You want to hit the fuel.
	**Squeeze the top handle or lever.** This depresses a button that releases the pressurized extinguishing agent in the extinguisher
	**Sweep from side to side** until the fire is completely out. Start using the extinguisher from a safe distance away, then move forward. Once the fire is out, keep an eye on the area in case it re-ignites.

Standard Operating Procedure (SOP) for Transport of Laboratory Waste

SOP no and version	11
SOP name	Standard Operating Procedure for transport of laboratory waste

Purpose: A standardized procedure for for Biosafety and Infection control during transport of laboratory waste

Logistics/ Materials required:

1. Utility gloves
2. Gown (Plastic)
3. Mask
4. Shoe (Gumboot)
5. Cleanable trolley

Responsibilities:

All laboratory personnel

Procedure for transportation of waste for disposal:

o Transportation to the autoclave room

a. Use easily cleanable trolley for transportation of waste to autoclave room
b. Do not touch the content of any bin or container. Handle out side surfaces only
c. Use service lift (where available) for transportation of waste between floors; if service is unavailable, then use passenger lift but do not allow any other individuals to enter the lift with you
d. If autoclave is unavailable for period of less than 24 hrs, keep the waste in laboratory. DO NOT LEAVE THE WASTE UNATTENDED IN ANY OTHER LOCATION
e. If autoclave is unavailable for period of more than 24 hrs, seal, tag the waste and keep in cold room

- **Transportation to the disposal site:**

Hand over the decontaminated/ autoclaved waste to the available facilities (incineration, landfill etc)

Standard Operating Procedure (SOP) for Receiving Specimen in the Laboratory

SOP no and version	**12**
SOP name	Standard Operating Procedure for receiving specimen in the laboratory

Purpose: A standardized procedure for for Biosafety and Infection control during receiving specimen.

Logistics/ Materials required:

1. Gloves
2. Gown (Plastic)
3. Mask
4. Shoe (Gumboot)

Responsibilities:

All laboratory personnel

Procedure for specimen receive :

1. Designate a particular room or area
2. Use necessary PPE
3. Check for proper labeling
4. Check for any leakage or damage
5. Check for appropriate information regarding specimen (Log and data sheet)
6. Deliver the specimen to respective section
7. Dispose used PPE properly

**NB: For any leakage or damage, contact designated persons.*
For specimens containing suspected highly infectious pathogen, contact with designated persons

Chapter V: Appendices

Appendix I

Biosafety laboratories:

* **Biosafety Level 1 (BSL1) Laboratory:**

This applies to the basic laboratory that handles agents (Table No.1) requiring biosafety level (BSL1). BSL1 requires no special design features beyond those suitable for a well-designed and functional laboratory. Biological safety cabinets (BSCs) are not required. Work is done on an open bench top, and containment is achieved through the use of standard precaution. In Bangladesh, all district and upazilla level laboratories are BSL1 standard laboratory.

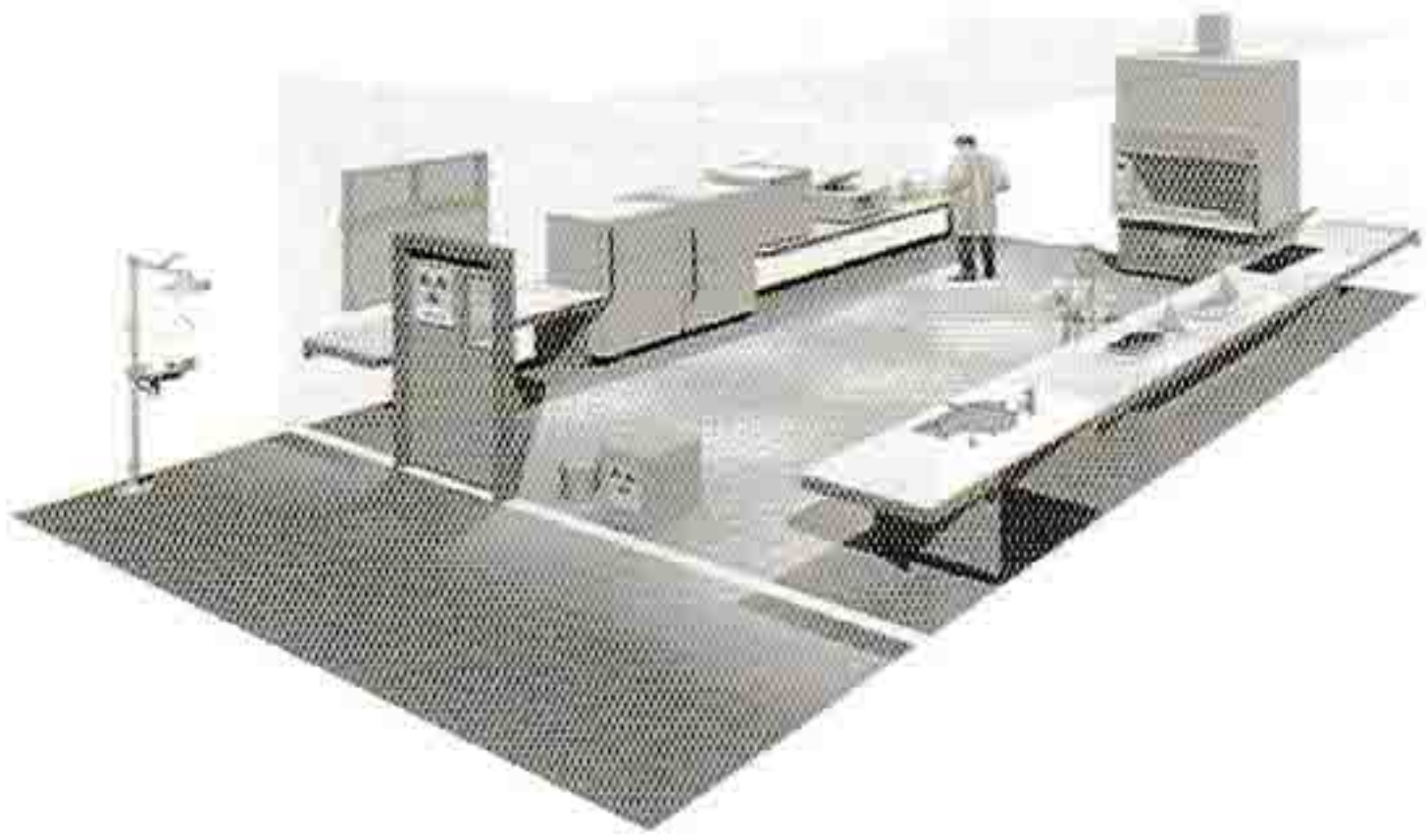


Fig: Biosafety level 1 laboratory

* **Biosafety Level 2 (BSL2) Laboratory:**

This applies to the laboratory that handles agents (Table No.1) requiring biosafety level (BSL2). In a BSL2 laboratory BSCs, centrifuge machines with sealed rotors or safety cups, appropriate personal protective equipment (i.e., gloves, laboratory coats, protective eyewear etc)

are used. To minimize environmental contamination hand washing sinks and decontamination facilities (autoclaves) are used in this laboratory. In Bangladesh, all medical college laboratories and some district level laboratories have BSL2 facilities.

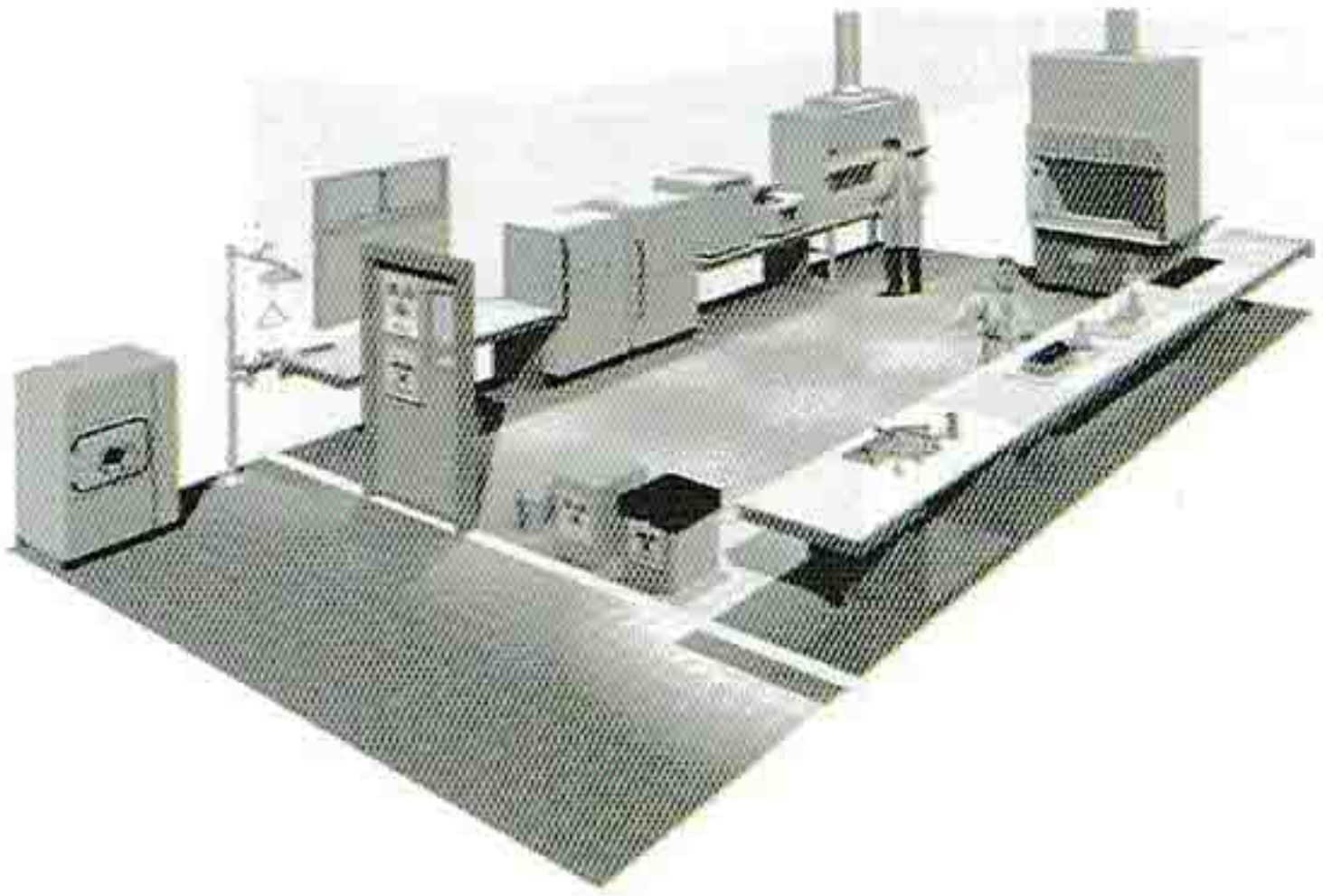


Fig: Biosafety level 2 laboratory

❊ Biosafety Level 3 (BSL3) laboratory

This applies to the laboratory that handles agents (Table no.1) requiring BSL3. To minimize the release of infectious organisms to adjacent laboratories and to the environment, BSL3 uses primary and secondary barriers such as suitable ventilation system (totally enclosed biological safety cabinet with negative pressure inside the room providing the highest possible level of protection to the personnel and the environment), appropriate respiratory protection, High-efficiency particulate air (HEPA) filtration of exhausted laboratory air and strictly controlled laboratory access. In Bangladesh, Institute of Epidemiology, Disease Control and Research (IEDCR), Bangladesh Institute of Tropical and Infectious Disease (BITID) and International Centre for Diarrhoeal Disease, Bangladesh (icddr,b) have BSL3 standard facilities.

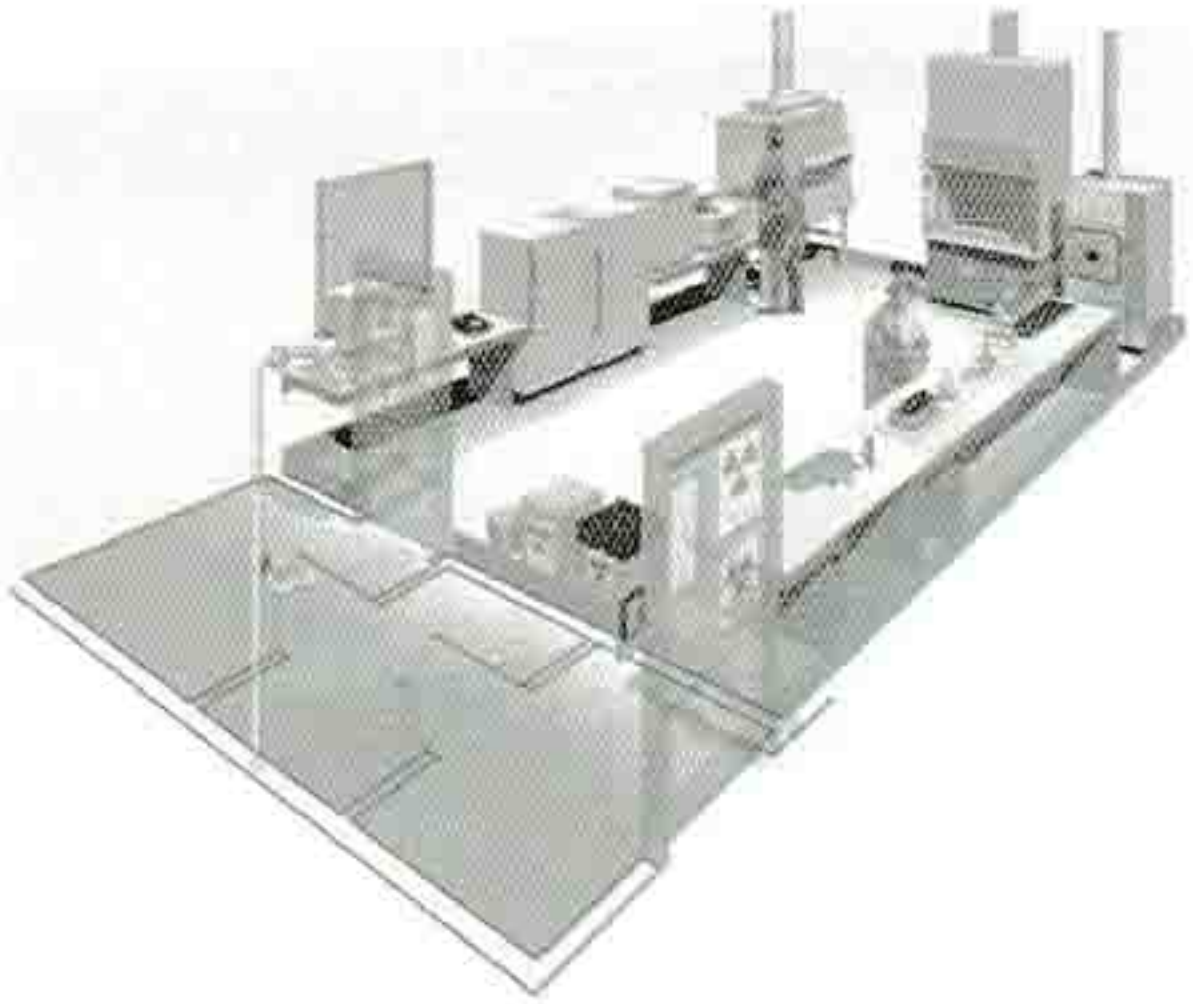


Fig: Biosafety level 3

* **Biosafety Level 4 (BSL4)**

This is the maximum containment available and is suitable for facilities manipulating agents (Table-1) requiring BSL 4. All activities in BSL 4 are confined to Class III biological safety cabinets, or Class II biological safety cabinets used with one-piece positive pressure personnel suits ventilated by a life support system. The facility itself is totally isolated from other lab and includes a specialized ventilation and waste management system. In Bangladesh there is no BSL 4 standard laboratory.

Fig: Biosafety level 4 laboratory

Appendix II

Commonly used specimen container

Stool collection container

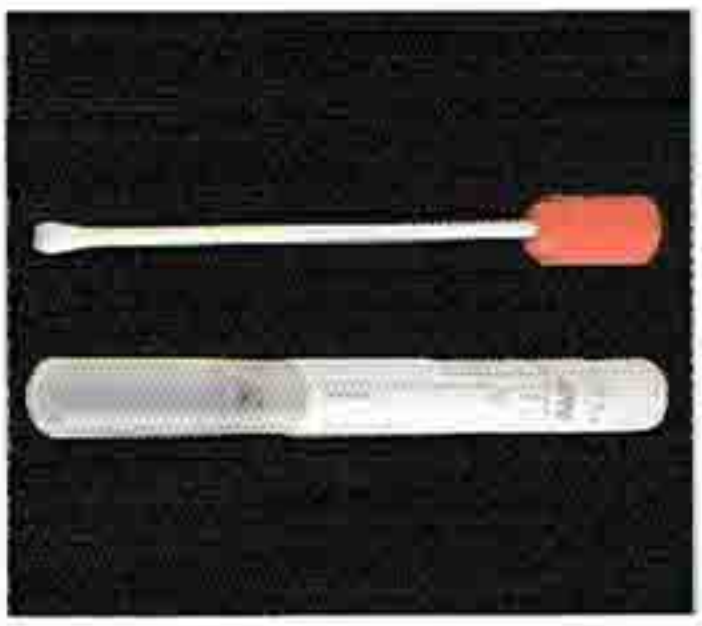
Urine collection container

Sputum collection container

EDTA tube

Vacutainer tube

Sodium heparin tube



Cary Blair media

Viral Transport Media

Appendix-III

List of laboratory equipments

1. Work surface
2. Lab floor
3. Pipette
4. Bio-safety cabinet
5. Centrifuge machine
6. Vortex mixture

List of disposable items

1. Pipette tips
2. Broken glass slides/cover slip
3. Falcon tube
4. Plastic transfer pipette
5. Syringe
6. Cotton swab
7. Alcohol pad
8. Test tube
9. Needle
10. ELISA plate
11. ICT strip
12. Used eppendrof tube
13. Disposable Petri dish
14. Pasteur pipette
15. Swab stick

List of reusable items

1. Test tube
2. Petri dish
3. Wire loop
4. Conventional blood culture bottle
5. Glass slide
6. Glass pipette
7. Freezer box
8. Test tube/ eppendrof rack
9. Cool box

Appendix-IV

WHO recommended colour-coding for health-care waste

Type of waste	Colour of container and markings	Type of container
Highly infectious waste	Yellow, marked "HIGHLY INFECTIOUS" 	Strong, leak-proof plastic bag
Other infectious waste, anatomical or pathologic waste	Yellow 	Leak-proof plastic bag or container
Sharps	Yellow, marked "SHARPS" 	Puncture-proof container
Chemical and pharmaceutical	Brown marked with a suitable symbol 	Plastic bag or container
Radioactive waste	———	Lead box, labelled with the radioactive symbol 
General health-care waste	Black	Plastic bag

Appendix-V

Preparation of commonly used chemical disinfectants

* **70% alcohol**
    1. Add 70 ml absolute ethanol (95%) to 30 ml water for preparation of 100 ml of 70% alcohol
* **Hypochlorite solution**-Two different dilutions of hypochlorite solutions are used for disinfection

- Hypochlorite solution (which contains 0.5% chlorine concentration), a disinfectant that is used to disinfect:
    1. Excreta
    2. Bodies
    3. Spills of blood/body fluids
    4. Vehicles and tires

- Hypochlorite solution (which contains 0.05% chlorine concentration) which is used to disinfect:
    1. Surfaces
    2. Medical equipment
    3. Bedding
    4. Reusable protective clothing before it is laundered
    5. Rinsing gloves between contact with different patients (if new gloves are not available)
    6. Rinsing gloves, aprons, boots before leaving a patient's room
    7. Disinfecting contaminated waste before disposal

Preparation of hypochlorite solution:

Using Liquid Bleach

1. Chlorine in liquid bleach comes in different concentrations. Any concentration can be used to make a dilute chlorine solution by applying the following formula:
2. [(% chlorine in liquid bleach) / (% chlorine desired)] – 1 = Total parts of water for each part bleach†
3. Example: To make a 0.5% chlorine solution from 3.5%† bleach:

4. [(3.5%) / (0.5%)] – 1 = 7 – 1 = 6 parts water for each part bleach
5. Therefore, add 1 part 3.5% bleach to 6 parts water to make a 0.5% chlorine solution.

Note:
"Parts" can be used for any unit of measure (e.g. ounce, litre or gallon) or any container used for measuring, such as a pitcher.

Using Bleach Powder

1. If using bleach powder, calculate the amount of bleach to be mixed with each litre of water by using the following formula:
2. [(% chlorine desired) / (% chlorine in bleach powder)] x 1 000 = Grams of bleach powder for each litre of water
3. Example: To make a 0.5% chlorine solution from calcium hypochlorite (bleach) powder containing 35% active chlorine:
4. [(0.5%)/ (35%)] x1 000 = 0.0143 x 1 000 = 14.3
5. Therefore, dissolve 14.3 grams of calcium hypochlorite (bleach) powder in each litre of water used to make a 0.5% chlorine solution.

Precautions during preparation of hypochlorite solution:

1. chlorine solutions gradually lose strength, and freshly diluted solutions must therefore be prepared daily;
2. clear water should be used because organic matter destroys chlorine;
3. 1:10 hypochlorite solution is caustic. Avoid direct contact with skin and eyes;
4. hypochlorite solutions give off chlorine. Prepare them in a well ventilated area;
5. use plastic containers for mixing and storing hypochlorite solutions as metal containers are corroded rapidly and also affect the hypochlorite

* Phenol-

1. Use at 2-5% v/v concentration
2. Do not keep diluted phenol for more than 24 hours

Appendix-VI

Commonly used methods for heat sterilization:

* **Autoclave** - here pressure is used to produce high temperature steam to achieve sterilization

    1. Place the material into the autoclave at 121°C for 15 minutes at a pressure of 15 lb/ in2
    2. Use brown tape (chemical indicator/autoclave tape) as indicator to see the efficacy of autoclave
    3. Use heat-resistant bacterium Geobacillus stearothermophilus as biological indicator if possible

* **Hot air oven** – electrical dry heat sterilizer
    1. Place the material in hot air oven at 160°C for 2 hours or 170 °C for 1 hour
    2. Dry the materials before placing into hot air oven since water will interfere with the process

Appendix-VII

List of microorganism according to risk group:

Risk group-1:

1. Micrococcus spp.
2. Lactobacillus spp.
3. Bacillus subtilis,
4. Common moulds and yeasts

Risk group-2:

Bacteria

1. Actinobacillus,
2. Actinomyces spp.
3. Bacillus cereus,
4. Bacteroides,
5. Bordetella pertussis,
6. Brucella spp.,
7. Campylobacter jejuni
8. Clostridium spp.
9. Corynebacterium diptheriae,
10. Diphtheroids,
11. Enterobacter,
12. E. coli,
13. Haemophilus ducreyi,
14. Helicobacter pylori
15. Klebsiella spp.
16. Legionella pneumophila,
17. Leptospira interrogans,
18. Listeria monocytogenes,
19. Moraxella spp.
20. Mycobacterium spp. - excluding M. tuberculosis, and members of the Mycobacterium tuberculosis complex (M. bovis, M. africanum, M. pinnipedii, M. microti, M. caprae, "Mycobacterium canettii")
21. Neisseria spp.
22. Pseudomonas spp.
23. Salmonella enterica spp.
24. Shigella spp.
25. Staphylococcus aureus,
26. Streptococcus pneumoniae,
27. Vibrio cholerae, serogroup O1, serogroup O139 (Bengal)

Virus

1. Adenovirus types 1, 2, 3, 4, 5 and 7
2. Coxsackievirus,
3. CMV,
4. Dengue fever virus,
5. Echovirus,
6. EBV
7. Haemophilus influenza,
8. Hepatitis A virus (HAV),
9. Hepatitis B virus (HBV),
10. Hepatitis C virus (HCV),
11. Hepatitis D virus,
12. Hepatitis E virus,
13. Herpes simplex virus,
14. Human Coronavirus (excluding SARS-Co-V),
15. Human papillomavirus,
16. Human Parainfluenza virus,
17. Human rotavirus,
18. Influenza virus type A (excluding 1918 influenza A (H1N1) strain and subtypes H5, H7 and H9),
19. Influenza virus (B and C)
20. Measles virus
21. Norwalk virus,
22. Parvovirus B19,
23. Respiratory syncytial virus,
24. Rhinovirus,
25. Rubella virus,
26. Vaccinia virus,
27. Varicella-zoster virus

Fungus

1. Candida albicans,
2. Cryptococcus neoformans,
3. Mycoplasma pneumonia

Parasite

1. Toxoplasma gondii,
2. Leishmania spp

Risk group-3

Bacteria

1. Chlamydophila psittaci
2. Rickettsia rickettsii
3. Mycobacterium tuberculosis and Mycobacterium tuberculosis complex (including M. bovis, M. africanum, M. pinnipedii, M. microti, M. caprae and M. canettii),
4. Bacillus anthracis

Virus

1. Chikungunya virus
2. Crimean- Congo haemorrhagic fever virus,
3. Eastern equine encephalitis (EEEV),
4. Western equine encephalitis (WEEV),
5. Hantavirus spp.
6. Human immunodeficiency virus (HIV),
7. Human T-lymphotropic virus (HTLV),
8. Influenza A virus subtypes H5, H7 and H9.
9. Yellow fever virus,
10. West Nile virus (WNV),
11. Vesicular stomatitis virus (VSV),
12. Severe acute respiratory syndrome (SARS) associated coronavirus,
13. Rabies virus

Fungus

1. Histoplasma capsulatum

Risk group-4

Virus

1. Ebola virus,
2. Japanese encephalitis virus,
3. Lassa virus,
4. Marburg virus,
5. Variola virus,
6. Nipah virus

Appendix-VIII

List of consultative group (Laboratory Expert)

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Design & Printing : Evergreen Printing and Packaging, Phone: 9568030