

Standard Operating Procedures of Laboratory Bio-safety for Infection Control in Nepal



Ministry of Health
Department of Health Services
NATIONAL PUBLIC HEALTH LABORATORY

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CHAPTER I: INTRODUCTION

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. Laboratory biosafety describes the containment principles, technologies and practices that are implemented to prevent the unintentional exposure to pathogens and toxins, or their accidental release. On other hand, 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.

National Guidelines on laboratory biosafety and biosecurity has been developed to ensure the safe handling of biohazardous materials in the laboratory and research facilities under the auspices of National Public Health Laboratory. The main focus of the guideline is the protection of faculty, staff and students. Ways to protect research and the environment are also considered.

Objective

The SOP has been developed:-

- To direct health laboratory staff to adapt biosafety/biosecurity practices at their respective workplaces thereby protecting laboratory people, community, environment and the laboratory/ biological materials.
- 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.

In Nepal, the most of clinical diagnostic laboratory are equipped with BSL1 standard laboratories. Central laboratory (NPHL), Patan academy of health sciences and few private diagnostic laboratories have both BSL1 and BSL2 facilities. BSL-1 and -2 laboratory facilities are sufficient to control most routine diagnostic work. The only BSL-3 lab is in National Public Health Laboratory (NPHL). BSL-3 and BSL-4 laboratories are beyond the scope of this SOP. Good Laboratory Practice (see annex-II) ensures biosafety and infection control in the laboratory.

CHAPTER II: STANDARD OPERATING PROCEDURE

Standard Operating Procedure for Laboratory house keeping

SoP no and version	01
SoP Name	Standard Operating Procedure for Housekeeping
Date of Finalization	

Purpose:

- To ensure a clean, safe and satisfying work environment.
- To ensure the availability of reagents, equipments and PPEs for biosafety.
- To provides work areas free of significant sources of contamination.
- To protect laboratory personnel from exposure of unnecessary incidents.

Requirements:

A. Equipment

- Temperature log sheet
- PPEs

B. Chemical/reagents

- Disinfectant
- Antiseptic floor cleaner
- Insecticides.

Responsibilities:

- Laboratory incharge

General instructions:

1. Work Environment

- a. Make up to date of availability of reagents for test procedure and PPEs needed for biosafety.
- 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.

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 (area)
- G. spill kits
- H. MSDS

*Emergency equipment must be properly maintained and regularly serviced.

Standard Operating procedures for Hand washing

SoP no and version	02
SoP Name	Hand Washing
Date of Finalization	

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:

Steps of Handwashing with soap and water

1. Wet hands with running water.



2. Apply the amount of product recommended by the soap manufacturer to hands, and rub hands together vigorously for at least 15 seconds, covering all surfaces of the hands and fingers.



3. Spread the leather over the backs of the hands.



4. Make sure that soap gets in between the fingers.



5. Grip the fingers on each hand.



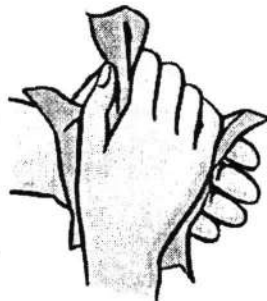
6. Pay particular attention to thumbs.



7. Press the fingertips into the palm of each hand.



8. Rinse your hands with water and dry thoroughly with clean towel.



With alcohol-based hand foam/gel rub

- Apply the amount of product recommended by the soap manufacturer to palm of one hand and rub hands together, covering all surfaces of hands and fingers, until hands are dry.

Note:

- a. Remove all jewelries (rings, bracelets) and watches*
- b. Ensure that the nails are clipped short*
- c. Roll the sleeves up to the elbow*

Standard Operating Procedure for Wearing, Removal and disposal of PPE

SoP no and version	03
SoP name	Wearing, Removal and disposal of PPE
Date of Finalization	

Purpose: Biosafety and infection control

Logistics/ Materials required:

- Gloves
- Aprons/lab coat
- Masks
- Closed toe footwear (Optional)

PPE wearing sequence

1. Lab coat/Apron

- Fully cover the body from neck to knees, arms to end of wrists and rap around the back.

- Fasten the buttons in front of lab coat.



2. Masks

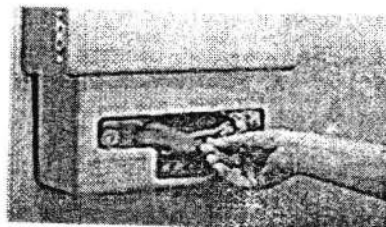
- Secure ties or elastic bands at middle of head and neck.
- Fit flexible bands to Nose Bridge.
- Fit comfortable to face and below chin.
- Fit check mask.



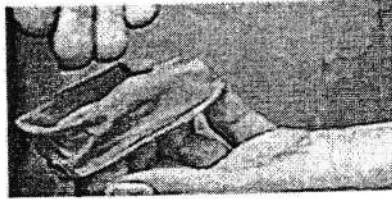
3. Gloves

Sequence of wearing gloves

- a. Select the appropriate size glove.



- b. Hold the glove at the opening, slip the fingers in to the glove and pull tight.



- c. With the gloved hand, hold the second glove at the opening and slip the ungloved fingers in to the glove and pull tight.



- d. Pull the gloves to the wrist of both hands.



4. Footwear

- Put on Closed toe footwear/laboratory shoe.

Sequence of removing PPE

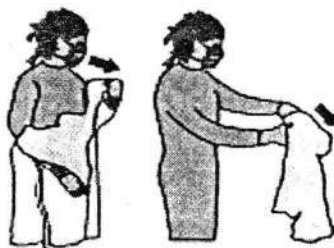
1. Gloves

- Don't touch the outside of gloves that are contaminated.
- Grasp the outside of glove with opposite gloved hand, and then peel off.
- Hold removed glove in gloved hand.
- Slide fingers of ungloved hand under remaining glove at wrist.
- Peel glove off over first glove.
- Discard gloves in waste container.



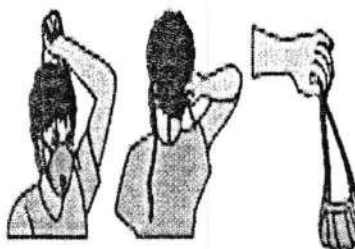
2. Lab coat/Apron

- Do not touch the outside of lab coat front and sleeves which are contaminated.
- Unfasten the buttons of lab coat.
- Turn lab coat inside out.
- Fold or roll into a bundle for disinfection process/autoclave



3. Mask

- Do not touch front of mask which is contaminated.
- Grasp the button and top of ties or elastics and remove.
- Discard in waste container.



Note: PERFORM HAND HYGIENE IMMEDIATELY AFTER REMOVING ALL PPE

Standard Operating Procedure during Specimen collection

SoP no and version	04
SoP name	Specimen collection
Date of Finalization	

Purpose:

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

Logistics required:

- Designated container
- Marker
- Bar code sticker
- Rack
- Tourniquet
- Disposable syringe, Swab stick
- Disposable tongue depressor
- Discard bin (Colour coded)
- Disinfectant
- Cool box
- PPEs
- Designated room/ corner

Responsibilities:

- Laboratory incharge
- Lab Technician
- Lab attendant
- Cleaner

General precaution for specimen collection:

- Instruct properly for patient preparation
- Check for the complete request form and confirm the identity of the patient.
- Check for the specimen container is labeled correctly.
- Check that the patient is appropriately prepared (e.g.- fasting, morning urine etc)
- Ensure that the specimen is collected properly
- Ensure that environmental and storage conditions are fulfilled to protect specimens from deterioration.
- Ensure the safe disposal of all materials used in specimen collection.
- Ensure that high risk specimens are identified and processed correctly.
- Ensure that all spillages and breakages are dealt with correctly.

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.

Proper handling of specimen from the time of collection through all stages of transportation, storage, and processing plays an important role in quality laboratory results.

For proper laboratory testing it is essential to select the appropriate container for specific test. Different sample types and their containers are kept in Annex-I and Table 1.

Standard Operating Procedure for Specimen Identification

SoP no and version	05
SoP name	Specimen Identification
Date of Finalization	

Purpose: To avoid significant risks that compromises the patient safety.

Procedure:

- Containers should be correctly labeled to facilitate identification.
- Label the containers with adhesive sticker (ID no, Name, Type of specimen and Date of collection) by permanent marker/Bar code generated sticker according to specimen request/specification form.
- Assign a separate series of laboratory remarks to each of designated sample types.

Laboratory Number Designations

REMARK	SECTIONS
HM	Hematology
BC	Biochemistry
EN	Endocrinology
MB	Microbiology
IM	Immunology
VR	Virology
P	Parasitology
HC	Histo-cytology

Standard Operating Procedures for Transportation of Specimen

SoP no and version	06
SoP name	Transport of specimen
Date of Finalization	

Purpose: To protect specimens and carrier during transportation

Logistics/ Materials required:

- PPEs
- Specimen log
- Declaration forms
- Shipment logs/tracking log
- Marker/ pen
- Basket to carry test tube racks/containers.
- Cold maintaining materials (ice packs, gel packs, dry ice, etc. as per the required shipment temperature)
- Triple packaging system
- Spill management kit
- Waste disposal bags

Responsibilities:

- Laboratory personnel in Charge
- Lab attendant

Procedure:

- Wash hands
- Wear PPEs

Transportation from sample collection site to testing site

- Seal tubes with parafilm or a tight fitting cap to contain the contents, keep test tubes in appropriate racks.

- Keep sample containers in zip lock plastic bags if possible.
- To avoid accidental leakage or spillage, secondary containers, such as boxes, should be used, fitted with racks so that the specimen containers remain upright.
- Carry sample containers, test tube racks in a proper basket/outer box acceptable only for within laboratory specimen transport.

Transportation within country

National shipment should follow the norms of international guidelines and comply with the triple packaging system (see annex-VI) locally adapted with best available materials.

Transportation outside country

- Sample is packed with basic triple packing system by well trained laboratory staff in IATA guidelines.
- Place all markings on packages 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.
 1. the shipper's (sender's, consignor's) name and address
 2. the telephone number of a responsible person, knowledgeable about the shipment
 3. the receiver's (consignee's) name and address
 4. temperature storage requirements
 5. When dry ice or liquid nitrogen is used: the technical name of the refrigerant, and the net quantity.
 6. Place specific labels for: 1. Biohazard sign for infectious substance, 2. Handling label for Orientation with the words 'THIS SIDE UP' or 'THIS END UP' should be present on the surface of the pack.

Note: Always ship the samples making sure that specimen reaches designated lab on a working day.

Standard Operating Procedure for storage of specimens

SoP no and version	07
SoP name	Storage of specimen
Date of Finalization	

Purpose: Biosafety and infection control

Logistics/ Materials required:

- PPE
- Freezer ($<0^{\circ}\text{C}$)
- Refrigerator ($2-8^{\circ}\text{C}$)
- Ultra low temperature freezer ($<-70^{\circ}\text{C}$)
- Liquid nitrogen tank (-196°C)
- Sample box
- Temperature log sheet
- Register book

Responsibility:

Laboratory in Charge

Procedures:

- Wear PPEs.
- Place the specimens in the designated pre-labeled box/Container.
- Place the box/ container in aforesaid cold system (as per required temperature).
- Do not keep different types of samples in same box/ container.
- Keep different types of samples in different designated locations of the refrigerator/ freezer.
- Keep the samples separately from reagents in different cold system (preferable)/ locations to prevent from mix up/ contamination.
- Maintain the temperature logs.
- Maintain inventory log.

Common specimen types and their storage facility

Specimen type	Storage facility
whole blood with anticoagulant	2-8 ⁰ c
Serum	-Do-
Swabs	-Do-
Stool	72 hrs at 2-8 ⁰ c
Urine	24 hrs at 2-8 ⁰ c
Sputum	24 hrs at 2-8 ⁰ c
Body fluids (Amniotic, abdominal, ascitis, synovial, bile, pericardial, pleural)	Plate as soon as received.
Abscess	24 hrs at room temperature.
CSF	6 hrs at 37 ⁰ c
Sample for CD4 count, viral load	-70 ⁰ c

Standard Operating Procedure for waste segregation

SoP no and version	08
Sop name	Waste segregation
Date of Finalization	

Responsibility

This SOP is applicable to all employees of the laboratory.

- Lab manager
- Lab personnel working in Laboratory
- Support staff

Procedure:

A. Non-contaminated (non-infectious) waste:

- These include papers, packaging boxes, plastic bags, hand paper towels, etc. That can be reused or recycled or disposed of as general, "household" waste.
- Place those waste in black or white color plastic bags inserted in plastic bucket.
- Replace with new plastic bag when it is full.

A. All contaminated (infectious) materials

- Do not store/place infectious wastes other than in the red bag in a red bin.
- Use double biohazard bags to seal used tubes, plastic Pasteur pipettes, tips, gloves, cotton, paper towels, specimen containers and cultures.
- Do not use but seal off the biohazard bag with tape when 3/4th full.
- Carefully transfer the bag to autoclave station.
- After autoclaving, bio hazard bags containing reusable lab ware are safe to open and clean and take non-reusable waste for incineration.

B. Sharps

Hypodermic needles, scalpels, knives and broken glasses; these should always be collected in puncture-proof containers fitted with covers and treated as infectious. Take the following precautions when collecting and segregating sharps—

- a. Do not mix sharps with papers or non-infectious wastes. Use properly labeled puncture-proof sharp container exclusively for SHARPS only.
- b. Discard used needles immediately into sharp container. Or use needle destroyer before discard.
- c. Pick broken glasses with the help of forceps/ receptacle and carefully put into sharp container.
- d. Do not use but send the sharp contents to autoclave when 3/4th full.

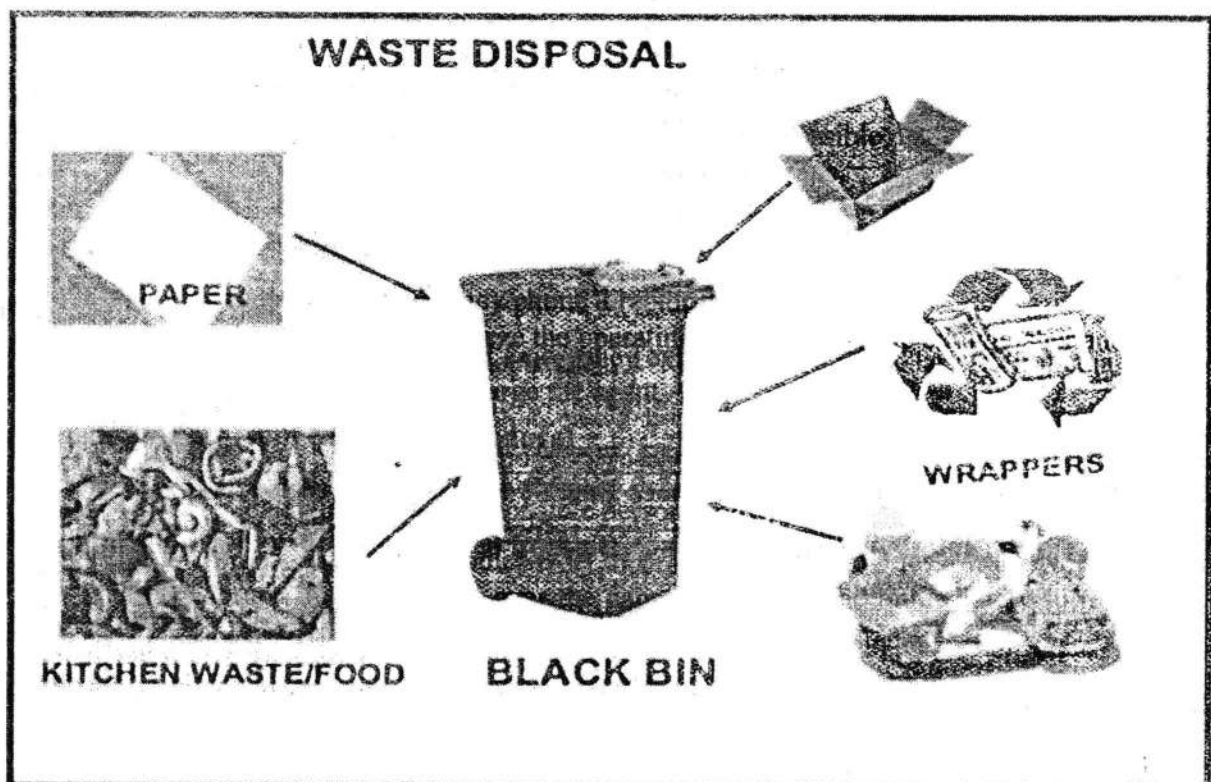
Note: Remove filled bags from the bins and replace them with respective color-coded new ones ready for the next day's lab work. After completion of the task, remove PPEs properly and perform hand-hygiene.

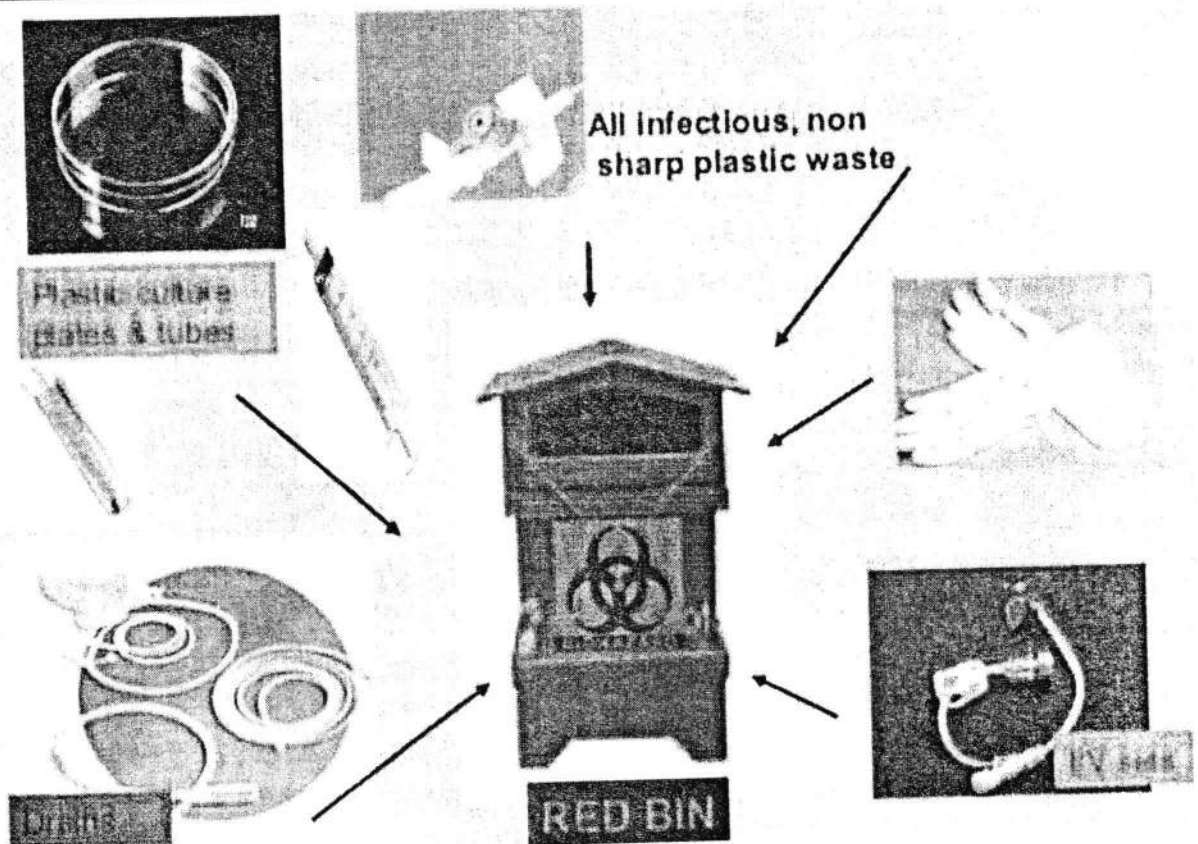
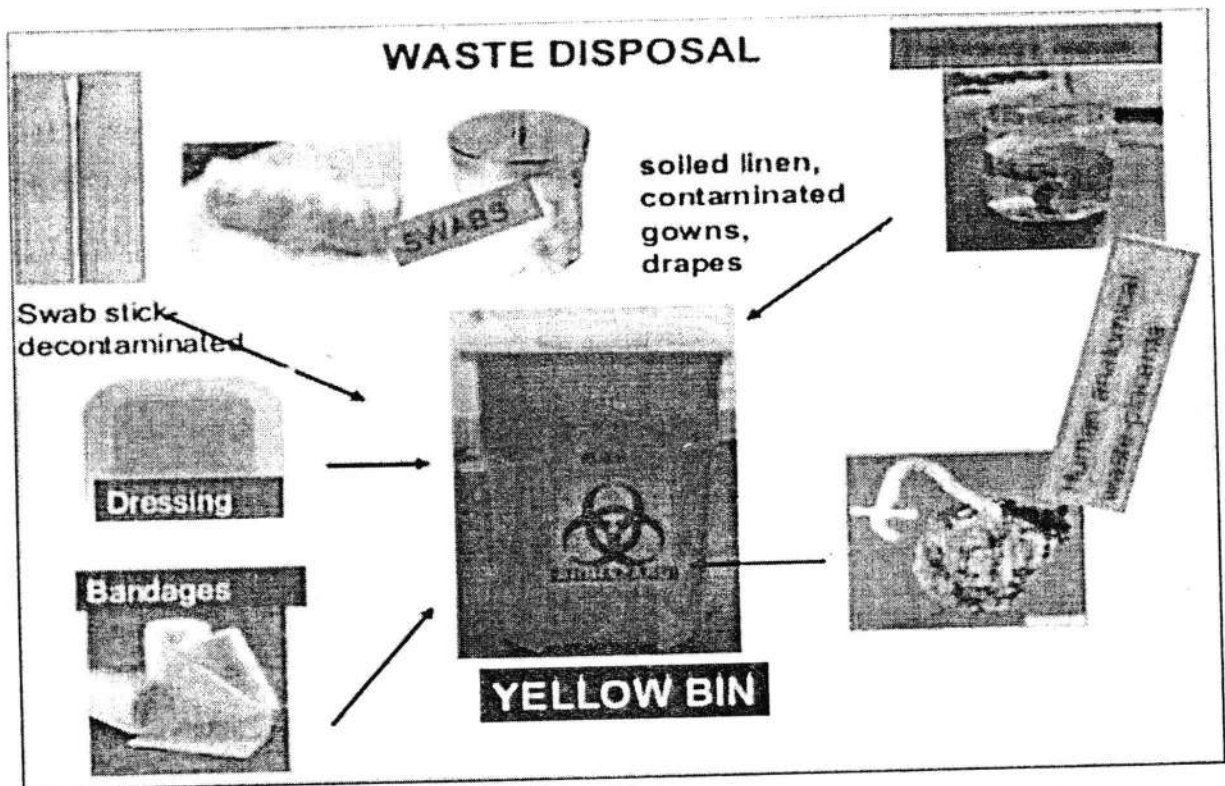
C. Liquid wastes (buffers, liquid from analyzers, liquid medium, etc.)

Acquire the following precautions when collecting and segregating—

- a. ENSURE THESE ARE NOT LIQUID CULTURE OR RADIO-ACTIVE SUBSTANCES. If liquid culture, treat as infectious one. For radio-active, follow the Table 2.
- b. Add liquid waste to bleach solution and leave for at least 30 minutes.
- c. Send to municipal waste system/ drain as appropriate.
- d. After completion of the task, remove PPEs properly and perform hand-hygiene.

Different color coded bin according to waste category





Standard Operating Procedure for transport of laboratory waste

SoP no and version	09
SoP name	Transport of laboratory waste
Date of Finalization	

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

Logistics/ Materials required:

- Utility gloves
- Gown (Plastic)
- Mask
- Shoe (Gum boot)
- Cleanable trolley

Responsibilities:

All laboratory personnel

Procedure for transportation of waste for disposal:

1. Wear PPEs (Gloves, utility gloves, gown, water proof boot if available)
2. Do not touch the content of any bin or container, handle the outer surface only.
3. Transport the waste carefully (use a cleanable trolley if available).
4. When using lift, do not allow any public inside till transportation is complete.
5. If autoclave is unavailable for <24hrs, bring back the waste and keep it in the lab, but NEVER leave it unattended anywhere else.
6. After completion of the task, remove PPEs properly and perform hand-hygiene.

Standard Operating procedures for waste treatment and Disposal

SoP no and version	10
SoP name	Waste treatment and Disposal
Date of Finalization	

Procedure:

1. Dispose materials in black colored waste bin/bag as general municipal waste. Further segregation of general wastes can be done as combustible and non-combustible; degradable and non-degradable; recyclable and non-recyclable.
2. Dispose materials in red colored waste bin/bag only after autoclaving.
3. Dispose materials in yellow container for “sharps” only after autoclaving.

Laboratory waste management should be done according waste category (See Table 2) to ensure bio-safety in laboratories and environment.

Note: “Contaminated material for re-use (glassware’s, etc.)” must be sterilized by autoclaving before use. After autoclaving the biohazard bags (containing reusable items) are safe to open and further clean for re-use. For autoclaved non-reusable wastes, follow hospital waste management guideline after decontamination of wastes (see annex V).

IMPORTANT NOTE: Treat the radio-active chemicals contaminated materials (e.g. gel with Ethidium bromide), if any, with charcoal before disposing.

Standard Operating Procedure for emergency management in laboratory

SoP no and version	11
SoP name	Emergency management in laboratory
Date of Finalization	

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

Preparedness for an emergency:

- Keep an adequately equipped emergency response supplies i.e. Spill Kit, First Aid Kit and Antidote, including Safety Shower/Eyewash area and fire extinguisher in common place of the laboratory that is known and easily accessible to all staffs related to laboratory works.
- Certify fire extinguisher annually.
- Maintain a department emergency phone directory.
- Know the building's floor plan & emergency exit routes.

Spill management (spill clean-up procedure)

1. Wear gloves, masks and protective clothing as required.
2. Cover the spill with cloth or paper towels to contain it.
3. Pour an appropriate disinfectant over the paper towels and the immediately surrounding area (generally, 5% bleach solutions are appropriate).
4. Apply disinfectant beginning at the outer margin of the spill area, toward the center.
5. After 30 minutes, clear away the materials. If there is broken glass or other sharps, use a dustpan to collect the material by forceps and deposit it into a puncture-resistant container for disposal.
6. Clean and disinfect the area of the spillage (if necessary, repeat steps 2–5).
7. Dispose of contaminated materials into a leak-proof, puncture-resistant waste disposal container.
8. After successful disinfection, inform that it has now been decontaminated.
9. Report significant spills/accidents immediately to the lab supervisor/ head using the incident reporting form and maintain the written records.

Additional notes:

- If dustpans are used to clear away the broken material, they should be autoclaved or placed in an effective disinfectant.
- Cloths/paper towels/swabs used to clean up should be placed in a red waste bin.
- If lab forms/printed/written materials are contaminated, the information should be copied onto another form (preferably snap-shot); and the original one discarded into the red colored waste bin.

Injury management

All injuries are required to be reported. Follow the procedures below in case of any of the listed events.

a. Biological Hazard Exposure to Eyes

- Flush the eyeball and inner eyelid with tepid water for 15 minutes. Forcibly hold the eye open to wash thoroughly behind the eyelids
- Contact local first aider to get medical attention promptly.

b. Biological Hazard Ingestion and Inhalation

- Contact local first aider to get medical attention promptly.

c. Biological Hazard Exposure on Skin

- Immediately flood the contaminated area with sufficient running water.
- Wash area with soap and water and apply 70% ethanol or a suitable antiseptic solution. Use paper towels to scrub affected area.
- Remove all contaminated clothing.
- Continue to rinse with cold water for 15 minutes. Again, wash contaminated areas with the water and disinfectant solution but do not apply creams or lotions.

In case of **needle-prick injuries**, wash the site well with soap and water and also immediately seek counseling for possible Post-exposure prophylaxis (PEP).

Fire/ explosion:

- Activate fire alarm.
- For small-scale fire, manage with fire blanket/ fire extinguisher.
- For major fire, protect yourself first, and escape as soon as possible.

- Do not use elevator, use stairways to escape.
- Assemble in the designated area outside.
- Report to authority and document it.

How to Use a Fire Extinguisher



	• Pull the pin that will break the seal and • test the extinguisher
	• Aim at the base of fire and • Ensure you have means of escape.
	• Squeeze the operating handle to operate extinguisher and discharge the agent
	• Sweep from side to side to completely extinguish the fire.

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.

Natural disaster (earthquake, flooding, land-slide, storm, etc.)

- Protect yourself first.
- Inform others.
- Assemble in the designated area outside.
- Report to authority and document it.

CHAPTER III: BIOSAFETY LABORATORIES

➤ Biosafety level 1 (BSL 1) laboratory:

This applies to the basic laboratory. BSL-1 requires no special design features beyond those suitable for a well-designed and functional laboratory. Biological safety cabinets (BSCs) are not required. Work may be done on an open bench top, and containment is achieved through the use of practices normally employed in a basic microbiology laboratory.

➤ Biosafety level 2 (BSL 2) laboratory:

This applies to the laboratory that handles agents requiring containment level 2. Agents requiring CL2 facilities are not generally transmitted by airborne routes but care must be taken to avoid the generation of aerosols. Primary containment devices such as BSCs and centrifuges with sealed rotors or safety cups are to be used as well as appropriate personal protective equipment (i.e., gloves, laboratory coats, protective eyewear). As well, environmental contamination must be minimized by the use of hand-washing sinks and autoclaves.

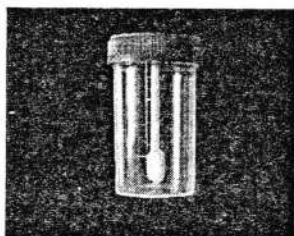
➤ Biosafety level 3 (BSL 3) laboratory:

This applies to the laboratory that handles agents requiring containment level 3. These agents may be transmitted by the airborne route, often have a low infectious dose to produce effects and can cause serious or life-threatening disease. Additional features to prevent transmission of CL3 organisms are appropriate respiratory protection, HEPA filtration of exhausted laboratory air and strictly controlled laboratory access.

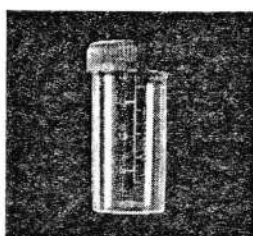
➤ Biosafety level 4 (BSL 4) laboratory:

This is the maximum containment available and is suitable for facilities manipulating agents requiring containment level 4. These agents have the potential for aerosol transmission, often have a low infectious dose and produce very serious and often fatal disease; there is generally no treatment or vaccine available. This level of containment represents an isolated unit, structurally independent of other areas. CL4 emphasizes maximum containment of the infectious agent by complete sealing of the facility perimeter with confirmation by pressure decay testing; isolation of the researcher from the pathogen by his or her containment in a positive pressure suit or containment of the pathogen in a Class III BSC line; and decontamination of air and other effluents produced in the facility.

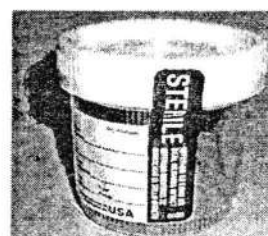
ANNEX I: COMMONLY USED SPECIMEN CONTAINER



Stool container



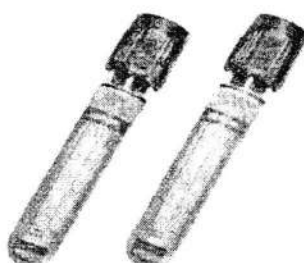
Urine container



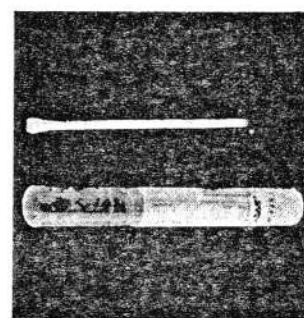
Sputum container



EDTA Tube



Vacutainer



Clary Blair media

ANNEX II: 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:

- I. Wear laboratory shoes
- II. Wash hands
- III. Wear laboratory coat/ gown
- IV. Do not eat, drink or smoke
- V. Do not store food, drinking water or beverage in the refrigerators
- VI. Do not use cosmetics
- VII. Do not wear ornaments (e.g. earring, nose pin, bracelet, ring etc.)
- VIII. Maintain nail hygiene
- IX. Cover hair and beard

- X. Avoid work with open cuts and broken skin (if required, cover it with bandage)
- XI. Take necessary vaccine(s) (HBV, TB, Rabies, flue, Typhoid vaccine etc)
- XII. Inform the laboratory in-charge in case of any emergency
- XIII. Do not attend mobile phone during work
- XIV. Avoid mouth pipetting

ANNEX III: WHO RECOMMENDED COLOUR-CODING FOR HEALTH-CARE WASTE

Type of waste	Colour of container and marking	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

ANNEX IV: COMMONLY USED METHODS FOR HEAT STERILIZATION

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

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

Hot air oven – electrical dry heat sterilizer

- Place the material in hot air oven at 160 °C for 2 hours or 170 °C for 1 hour
- Dry the materials before placing into hot air oven since water will interfere with the process

ANNEX V: 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.

a. Laboratory and laboratory equipments:

- Laboratory premises should keep clean and decontaminate regularly (SOP.).
- Every day 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.
- Clean laboratory floor with phenol or Lysol regularly.

i. Laboratory disposable items:

- All infectious materials and disposable items should be decontaminated before being washed and stored or discarded. Autoclaving is the preferred method and each individual working with biohazardous material is responsible for its decontamination before disposal.
- 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.

ii. 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.

b. List of laboratory equipments

- Work surface
- Lab floor
- Pipette
- Bio-safety cabinet
- Centrifuge machine
- Vortex mixture

List of disposable items

- Pipette tips
- Broken glass slides/cover slip
- Falcon tube
- Plastic transfer pipette
- Syringe
- Cotton swab
- Alcohol pad
- Test tube
- Needle
- Used eppendorf tube
- Disposable Petri dish
- Pasteur pipette
- Swab stick
- Pasteur pipette
- Swab stick

List of reusable items

- Test tube
- Petri dish
- Wire loop
- Conventional blood culture bottle
- Glass slide
- Glass pipette
- Freezer box
- Test tube/ eppendorf rack
- Cool box

ANNEX VI: BASIC TRIPLE PACKING SYSTEM

Primary Receptacle

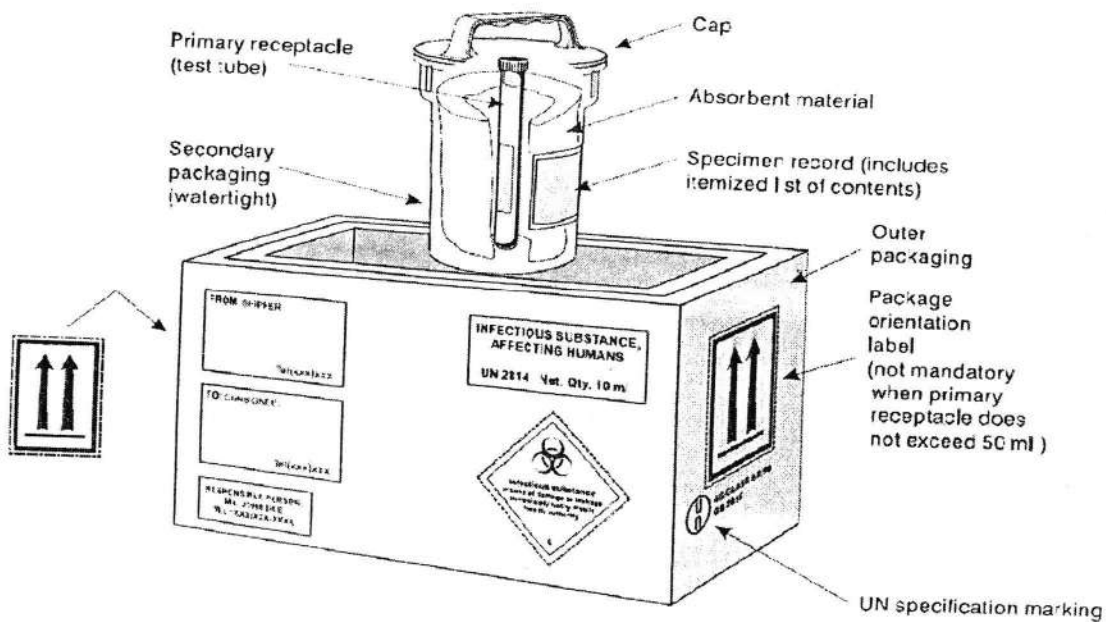
- Water-tight, leak-proof, container with specimen.
- To be packaged with absorbent material to contain potential leakage.

Secondary Packaging

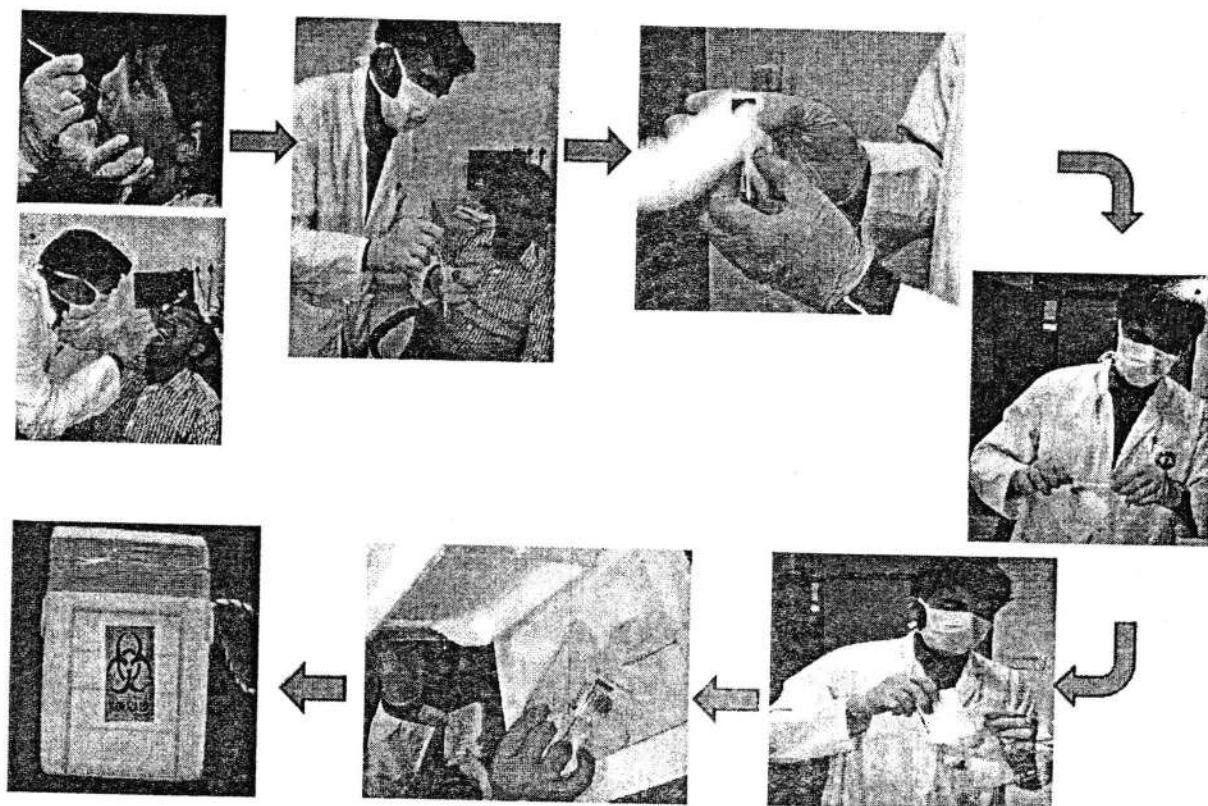
- Durable, water-tight, leak-proof packaging, to protect primary receptacle.
- May hold several primary receptacles, but with sufficient absorbent material.
- Ice or dry ice, if any, to be placed outside the secondary package.

Outer Packaging

- Of adequate strength for capacity, mass and intended use, with minimum dimensions of 10 cm x 10 cm.



Specimen Packaging



ANNEX VII: LIST OF MICROORGANISM ACCORDING TO RISK

GROUP

Risk group-1:

- *Micrococcus* spp.
- *Lactobacillus* spp.
- *Bacillus subtilis*,
- Common moulds and yeasts

Risk group-2:

- | | |
|--|---|
| • <i>Actinobacillus</i> , | • <i>Listeria monocytogenes</i> , |
| • <i>Actinomyces</i> spp. | • <i>Moraxella</i> spp. |
| • <i>Bacillus cereus</i> , | • <i>Mycobacterium</i> spp. - excluding <i>M.</i> |
| • <i>Bacteroides</i> , | tuberculosis, and members of the |
| • <i>Bordetella pertussis</i> , | <i>Mycobacterium tuberculosis</i> |
| • <i>Brucella</i> spp., | complex (<i>M. bovis</i> , <i>M. africanum</i> , |
| • <i>Campylobacter jejuni</i> | <i>M. pinnipedii</i> , <i>M. microti</i> , <i>M. caprae</i> , |
| • <i>Clostridium</i> spp. | " <i>Mycobacterium canettii</i> ") |
| • <i>Corynebacterium diphtheriae</i> , | • <i>Neisseria</i> spp. |
| • Diphtheroids, | • <i>Pseudomonas</i> spp. |
| • <i>Enterobacter</i> , | • <i>Salmonella enterica</i> spp. |
| • <i>E.Coli</i> , | • <i>Shigella</i> spp. |
| • <i>Haemophilus ducreyi</i> , | • <i>Staphylococcus aureus</i> , |
| • <i>Helicobacter pylori</i> | • <i>Streptococcus pneumonia</i> , |
| • <i>Klebsiella</i> spp. | • <i>Vibrio cholerae</i> , serogroup O1, |
| • <i>Legionella pneumophila</i> , | serogroup O139 (Bengal) |
| • <i>Leptospira interrogans</i> , | |

Virus

- Adenovirus types 1, 2, 3, 4, 5 and 7
- Coxsackievirus,
- CMV,
- Dengue fever virus,
- Echovirus,
- EBV
- *Haemophilus influenza*,
- Hepatitis A virus (HAV),
- Hepatitis B virus (HBV),
- Hepatitis C virus (HCV),
- Hepatitis D Virus,
- Hepatitis E virus,
- Herpes simplex virus,
- Human Coronavirus (excluding SARS-Co-V),
- Human papillomavirus,
- Human Parainfluenza virus,
- Human rotavirus,
- Vaccinia Virus,
- Influenza virus type A (excluding 1918 influenza A (H1N1) strain and subtypes H5, H7 and H9),
- Influenza virus (B and C)
- Measles virus
- Norwalk virus,
- Parvovirus B19,
- Respiratory syncytial virus,
- Rhinovirus,
- Rubella virus,
- Varicella-zoster virus

Fungus

- *Candida albicans*,
- *Cryptococcus neoformans*,
- *Mycoplasma pneumonia*

Parasite

- *Toxoplasma gondii*,
- *Leishmania spp*

Risk group-3

Bacteria

- *Chlamydia psittaci*
- *Rickettsia rickettsii*
- *Mycobacterium tuberculosis* and *Mycobacterium tuberculosis* complex (including *M. bovis*, *M. africanum*, *M. pinnipedii*, *M. microti*, *M. caprae* and *M. canettii*),
- *Bacillus anthracis*

Virus

- Chikungunya virus
- Crimean- Congo haemorrhagic fever virus,
- Eastern equine encephalitis (EEEV),
- Western equine encephalitis (WEEV),
- Hantavirus spp.
- Human immunodeficiency virus (HIV),
- Human T-lymphotropic virus (HTLV),
- Influenza A virus subtypes H5, H7 and H9.
- Yellow fever virus,
- West Nile virus (WNV),
- Vesicular stomatitis virus (VSV),
- Severe acute respiratory syndrome (SARS) associated coronavirus, ,
- Rabies virus

Fungus

- *Histoplasma capsulatum*

Risk group-4

Virus

- Ebola virus,
- Japanese encephalitis virus,
- Lassa virus,
- Marburg virus,
- Variola virus,
- Nipah virus

ANNEX VIII: PREPARATION OF COMMONLY USED CHEMICAL DISINFECTANTS

➤ 70% alcohol

- Add 70 ml absolute ethanol (95%) to 30 ml water for preparation of 100 ml of 70% alcohol.

➤ Bleach-Two different dilutions of bleach are used for disinfection.

- 1:10 bleach solution (which contains 0.5% chlorine concentration), a strong disinfectant that is used to disinfect:
 - Excreta - Bodies - Spills of blood/body fluids
 - Vehicles and tires
 - It is also used to prepare 1:100 bleach solution
- 1:100 bleach solution (which contains 0.05% chlorine concentration) which is used to disinfect:
 - Surfaces - Medical equipment
 - Bedding
 - Reusable protective clothing before it is laundered

Also recommended for:

- Rinsing gloves between contact with different patients (if new gloves are not available Rinsing gloves, aprons, boots before leaving a patient's room)
- Disinfecting contaminated waste before disposal
- To prepare 1:10 bleach solution add one volume of household bleach (e.g. 1 litre) to nine volumes of clean water (e.g. 9 litre).
- To prepare 1:100 bleach solution add one volume of 1:10 bleach solution (e.g. 1 litre) to nine volumes of clean water (e.g. 9 litre).

Note: 1:100 bleach solution can also be prepared directly from household bleach by adding 1 volume of household bleach to 99 volumes of clean water (e.g. 100 ml of bleach to 9.9 litres of clean water) but making it up from 1:10 bleach solution is much easier).

When preparing chlorine solutions for use note that:

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

Phenol-

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

TABLE 1.DIFFERENT TYPES OF SPECIMEN AND THEIR CONTAINERS

Specimen	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 screw 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 stick swab (Cotton/Dacron/Alginate- where applicable) in test tube Blister

Note: For culture, specimen's container must be sterile, for molecular test specimen container must be nuclease free.

Table 2: LABORATORY waste management according to category

Waste Category	Description and Examples	Management
Infectious/ contaminated waste	Non degradable 1.Sharps Hypodermic needles, scalpels, broken glassware & plastic ware, blades	Segregation -Puncture proof colour coded container -If colour container not available then use any beaker/ bucket containing 0.5% hypochlorite solution Processing Before disposal Sterilize by Autoclave / 0.5% hypochlorite solution Transport to the place of disposal Solid-Cleanable trolley Liquid-Bucket with Lid Disposal Incineration/Land fill
	2.Non Sharps Syringes, infusion sets, ELISA plate, ICT, micro centrifuge tube, Petri dish, Vacutainer, plastic tube, pipette tips, masks, gloves	Segregation - colour coded container - If colour container not available then use any beaker/ bucket containing 0.5% hypochlorite solution Processing Before disposal Sterilize by Autoclave / 0.5% hypochlorite solution Transport to the place of disposal Cleanable trolley Disposal Incineration/Land fill
	3.Degradable Blood, Serum, Plasma, Urine, Stool, Swab, Body fluids, Tissues and organs	Segregation - colour coded container - If colour container not available then use any beaker/ bucket containing 0.5% hypochlorite solution Processing Before disposal Sterilize by Autoclave / 0.5% hypochlorite solution Transport to the place of disposal Cleanable trolley

		Disposal Incineration/Land fill
Non infectious household waste	Tissue paper, Paper	Segregation -colour coded container . Processing before disposal -None Transportation to the place of disposal Cleanable trolley Disposal Incineration/landfill/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 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	Keep in separate, sealable container with the labeling of chemical waste Dilute strong acid and base into neutral pH and disperse into sewerage system 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 ug/ml- release into drain >10 ug/ml- charcoal filtration for deactivation
Waste 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 substances	Follow individual laboratory's own systems 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**



BIOHAZARD

ADMITTANCE TO AUTHORIZED PERSONNEL ONLY

