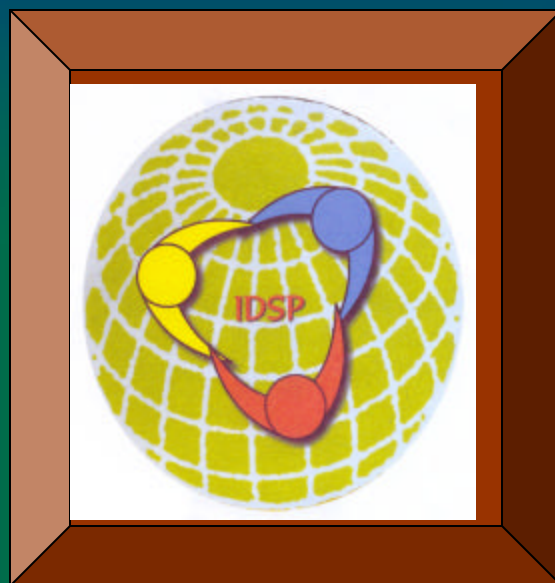


Integrated Disease Surveillance Project INDIA



BIOSAFETY MANUAL For Public Health Laboratories



Government of India
Directorate General of Health Services
Ministry of Health and Family Welfare
Nirman Bhawan , New Delhi

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Abbreviations

BMW	-Bio medical Waste
BSC	-Biosafety Cabinet
CHC	- Community Health Centre
DSO	-District Surveillance Officer
DSU	-District Surveillance Unit
GMT	-Good Medical Technique
GLP	- Good Laboratory Practice
CPCB	-Central Pollution Control Board
HCW	- Health Care Worker
HEPA	- High Efficiency Particulate Air
IDSP	- Integrated Disease Surveillance Project
PHC	- Primary Health Centre
PPE	- Personal Protective Equipment
PEP	- Post Exposure Prophylaxis

GLOSSARY

Autoinoculation - means self inflicted injury while handling sharp objects like needles etc.

Biomedical waste- Waste material generated in the hospital during diagnosis and treatment of patients.

Biosafety cabinet -cabinet designed to protect the operator, laboratory environment and work material from exposure to infectious aerosol.

Disinfection—destruction of vegetative forms of organisms which may or may not be pathogenic.

Sterilization - destruction of all forms of organisms including spores.

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

Universal Precautions- refers to precautions consistently used for all patients regardless of their blood borne infection status. -

Preface

Public health laboratories play a critical role in disease surveillance and response. They are involved in confirmation of etiological diagnosis during outbreaks and in prevention and control of diseases by keeping a watch on existing as well as emerging and re-emerging pathogens and environmental monitoring.

Laboratory bio-safety is an integral component of the overall laboratory quality assurance policy. An integrated approach to laboratory bio-safety, including containment of microbiological agents and toxins, safe handling and transport need to be an essential ingredient of all preparedness plans so as to minimize the possibility of laboratory acquired infections and resultant spread to the community, and promote public health.

This manual acknowledges the role of effective laboratory bio-safety controls and guidelines for laboratory practice at State & District level in order to manage the risks to laboratory workers and the community from microbiological agents and toxins.

Currently, bio-safety issues and bio-medical waste management at all levels of health care delivery and laboratory services are not explicitly prescribed and practiced. The written guidelines when available are not implemented due to the health personnel's ignorance, attitude and lack of training. With the rising HIV, Hepatitis B and C, incidence in the community, there is a gradual increase in the awareness of risk to the health personnel and practice of 'Universal precautions'. There is a feeling that universal precautions are not feasible and are expensive. This has resulted in reluctance, on the part of the health providers and Laboratory professionals at various levels, to participate in bio-safety programs.

This manual envisages to address bio-safety issues related to the health personnel and to the community as proposed in the Project Implementation Plan of IDSP. Laboratory Bio-safety manual is expected to be a helpful reference and guide and may be used in conjunction with Laboratory manual for PHC & District health labs.

The technical help extended by professionals of NICD, WHO India office and World Bank ,New Delhi for improvement of this manual is acknowledged.

Chapter - 1

General Principles of Biosafety and Code of Practice

1.1 What is Bio-Safety?

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

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

1.2 Code of Practice

This code is a listing of the most essential laboratory practices and procedures that are basic to good microbiological techniques. International standards are rigid and in the developing country like India, it is not always possible though not impossible to adhere to such guidelines. The most important concepts are listed below:



Fig 1. International biohazard warning symbol

1.2.1. Entry / access to laboratory area

- The international biohazard warning symbol (Fig 1) and sign must be displayed on the doors of the rooms where high risk microorganisms are handled.

- Entry to laboratory working area should be restricted only for laboratory persons.
- Doors of the laboratory should be kept closed.
- Children should not be allowed to enter laboratory working areas.

1.2.2. Laboratory Design and Facilities

- Enough space should be available
- Smooth easily cleanable walls, ceiling and floors impermeable to liquids and resistant to chemicals and disinfectants, should be preferred.
- Bench tops should be impervious to water and resistant to disinfectants.
- Ample illumination should be available for laboratory procedures.
- Storage space must be adequate to hold supplies for immediate use and to prevent overcrowding on bench tops .
- Regular, water supply should be available.
- Wash basins with running water, if possible, should be provided in the laboratory room preferably near the exit door.
- Suitably equipped first aid box should be available in the laboratory.
- Rodents and insects control procedure in the laboratory should be in place.

1.2.3. Universal precautions

It refers to precautions consistently used for all patients regardless of their blood borne Infection status. Under universal precautions, blood and body fluids of all patients are considered potentially infectious for HIV, HBV, HCV and other blood borne pathogens.

1.2.4. Universal precautions :

One of the fundamental principles of a biosafety program is the adherence to the practice of precaution. This may be defined as the minimal standard of work performance to prevent the exposure to pathogenic agents; and includes education, personal protective equipment, hand washing, and employing safe work practices .It requires the individual to assume the material infectitious and to act accordingly.

Barrier Protection should be used at all time to prevent skin and mucous membrane contaminated with blood and body fluid. The type of barrier protection used should be appropriate for the type of procedure which is being performed and the type of exposure anticipated.

Barrier protection: Protective barriers reduce the risk of exposure of the laboratory worker's skin or mucous membrane to potentially infective materials including blood and the body fluids.

Some of the important components of universal precautions are as under

1.2.4.1 Personal Protective Equipments

(A.) Gloves

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

Gloves should be:

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



Fig 2: Use of Gloves

Uses of Gloves

- Worn while collecting/handling blood specimens, blood soiled items or whenever there is a possibility of exposure to blood or body fluids containing blood. (Fig 2)
- Worn while disposing laboratory waste

When to change gloves

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

(B). Laboratory gowns

- Laboratory gowns or uniforms (preferably wrap-around gowns) should be worn when in the laboratory and should be removed before leaving.

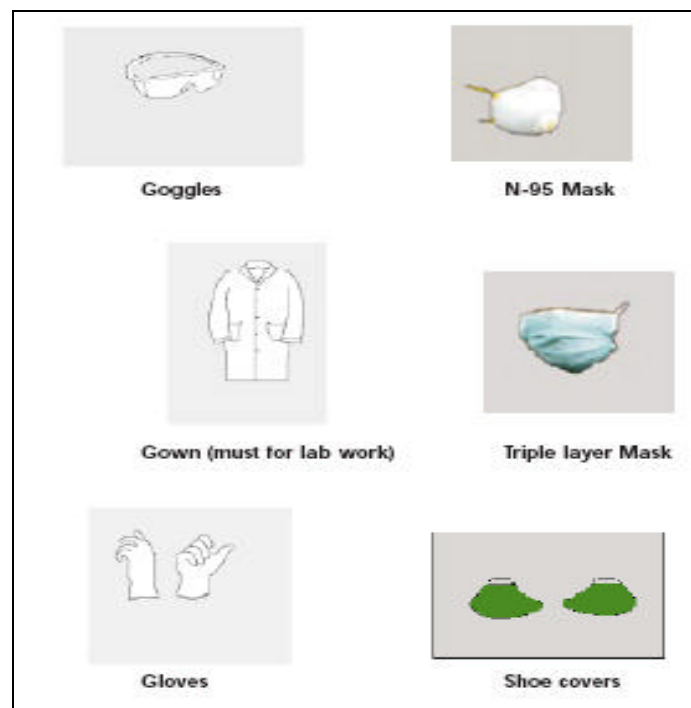
- Plastic aprons should be used while cleaning infected re-usable and during disposing wastes.

(C). Facial protection

- Simple and cheap deflector masks and protective glasses may be worn if splashing or spraying of blood/body fluids is expected.

(D).Occlusive bandage

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



Personal Protective Equipments
Figure 3

N-95 and triple layer mask is used while handling of patient's samples who are suspected of Avian influenza .Some common used PPE's are depicted in (Fig 3.)

.1.2.4.2 Hand washing

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

- The role of hands in the transmission of infections has been well demonstrated, and can be minimized with appropriate hand hygiene.
- Hands should be washed thoroughly in running water with soap without missing any area (Fig 4).

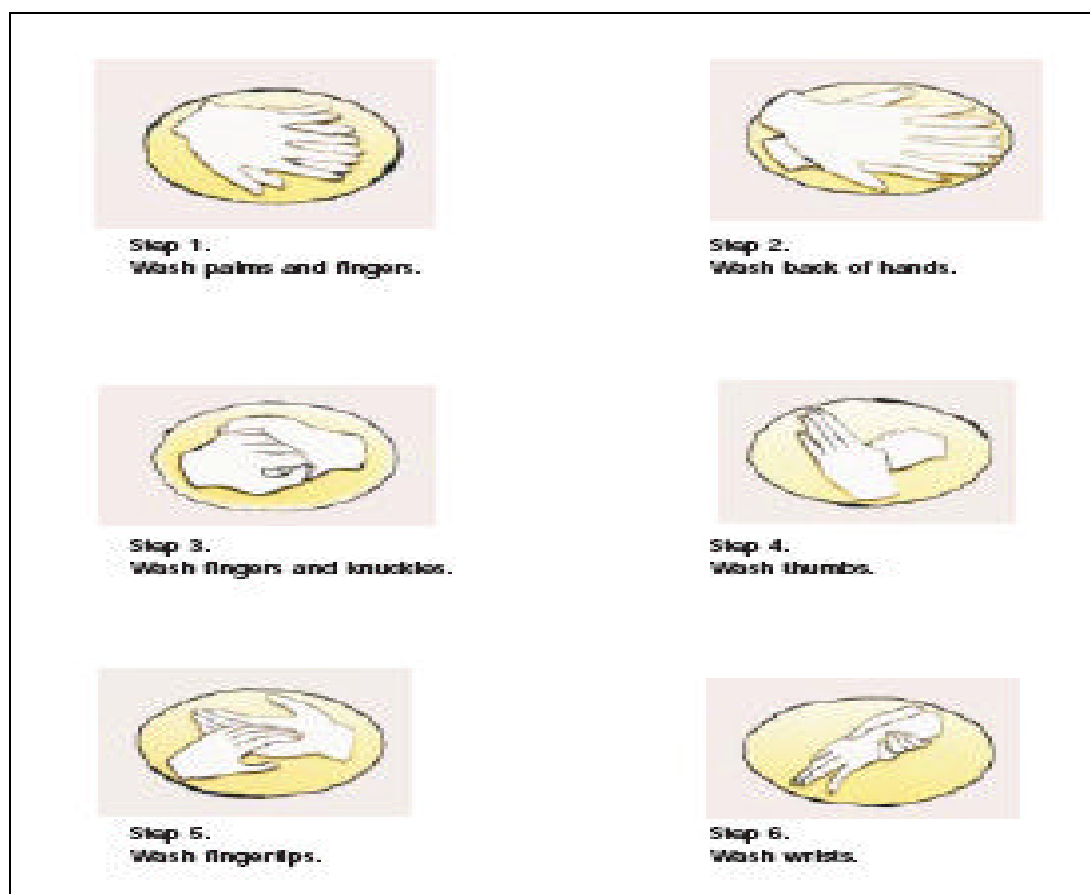


Fig 4: Steps for good hand washing

- Washing of hands is mandatory
- Wash immediately after
 - ✓ contamination with blood / body fluids
 - ✓ After removing gowns / coats and gloves
 - ✓ Before eating / drinking and leaving the laboratory

The following facilities are required;

- Running water: large washbasins preferably with hands free controls, which require little maintenance and with anti-splash devices.
- Products: dry soap or liquid antiseptic depending on the procedure. - Ideally, liquid soap dispensers should be provided to the laboratories, which should be regularly cleaned and maintained. If not feasible, soap bars after washing should be left in a dry tray to prevent contamination with some microorganisms which grow in moist conditions
- Suitable material for drying of hands; disposable towels, reusable sterile single use towels or roller towels which are suitably maintained.

Gloves should not be regarded as substitute for hand washing

1.2.4.3 Safe techniques:

- All procedures and manipulations of potentially infectious material should be performed in a separate area, to minimize the formation of droplets, aerosols, splashes or spills.
- Mouth pipetting should be strictly prohibited. Mechanical pipetting devices should be used for pipetting of all liquids in the laboratory. (Fig 5)
- Centrifugation should be done in tubes with safety caps.



Fig 5: Proper use of pipetting device

1.2.4.4. Safe handling of sharps

- Extreme care should be used to avoid auto-inoculation.
- All chipped or cracked glassware should be discarded in appropriate containers.
- Broken glass should be picked up with a brush and pan. (Fig 6) Hands must never be used.
- The disposable needles should never be manipulated, bent, broken, recapped or removed from the syringes.(Fig 7)

- The used sharps should never be passed directly from one person to another a kidney tray(Fig 8) may be used for this purpose
- Each Health Care Worker should dispose of his/her own sharps.
- Used needles should be discarded in puncture-proof rigid containers (plastic or cardboard boxes) after disinfection in 0.5-1% freshly prepared **sodium hypochlorite solution (common bleach)** only .Do not mix with other waste. If a needle shredder is available,(Fig 9) only the needles or the needles along with syringe nozzle may be shredded depending upon the type of the shredder
- Sharp disposable containers should be located close to the point of use.
- Sharp disposal containers should be sent for disposal when three-fourth full.



Fig 6: To pick broken glass use brush & pan or cardboard.



Fig 7: Do not Recap used needles



Fig 8: A Kidney tray may be used to pass the sharps



Fig 9: Needle Shredder

DO'S	DON'TS
Pass syringes and needles in a tray. Preferably cut it with needle cutter	Never pass syringes and needle directly to next person
Put needle and syringes in 2% hypochlorite solution if needle cutter is not available	Do not bend or break or recap used needle with hands
Remove cap of needle near the site of use	Never test the fineness of the needle's tip before use with bare or gloved hand
Pick up open needle from tray/drum with forceps	Never pick up open needle by hand
Destroy syringes by burning their tips/or if cutters not available	Never dispose it off by breaking it with hammer/stone

1.2.4.5. Safe handling of specimens

- Specimens, specially blood and body fluids should be collected in pre-sterilized screw-capped **plastic containers** properly sealed to prevent spillage or leakage.
- Pre-sterilized /autoclaved / disposable syringes and needles for venepuncture or lancets / cutting needles for finger prick should be used. Auto destructive syringes if available are good alternative.
- Cuts in hands should be properly covered with waterproof adhesive bandages.

- Disposable gloves should be worn while collecting blood / body fluids and proper asepsis should be maintained.
- If a sample shows evidence of breakage (in case not collected in the above container), leakage or soiling, it should be transferred with a gloved hand into a second sterile container. Any important information should be rewritten from the old to the new container. This should only be done if sample is highly precious otherwise it should be discarded and fresh sample to be collected.
- Do not keep samples on requisition forms.
- If the requisition slip is contaminated with blood, it should be rejected. In case of emergency, the contaminated slip may be handled using gloves.
- Hands should be thoroughly washed with soap and water before and after handling specimens.
- If the outside of the container is visibly **contaminated** with blood it should be cleaned with disinfectant. All blood specimens should be placed in small leak-proof impervious plastic tubes for transportation to the laboratory.

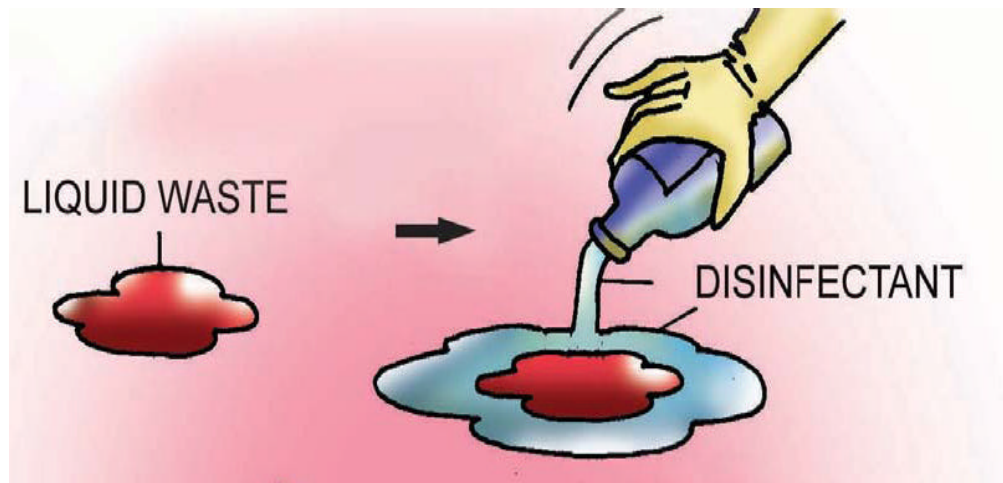
Safe handling of blood/body fluid spills

In case of a spill of blood / body fluid in the laboratory, the area should be flooded with a disinfectant solution. e.g. Freshly prepared 0.5-1% Sodium hypochlorite solution and left for 10 minutes (fig 10). After wearing gloves, the area should be covered with paper towels or gauze sponges to absorb the liquid followed by a thorough wash with soap and water. All contaminated materials should be disposed of as infectious waste.

Procedure to clean up spill

Procedure to clean up all spills	
⇒	Pour 1 % freshly prepared Sodium hypochlorite solution over spills in sufficient quantity.
⇒	Cover the spills with paper towel or absorbent materials.
⇒	Leave for 10 min.
⇒	Clean it
⇒	Wipe up the whole spill with fresh absorbent material using gloved hands and discard it in a contaminated waste container
⇒	Wipe the surface with soap and water.

Step 1



Step 2

Wait for 10 minutes and remove with help of Tissue or guage piece. Throw in yellow bag.

Step 3



Clean floor with Phenol or soap and water.

Fig 10: Handling of spills of blood/body fluids.

1.2.4.6. Laundry and linen:

Although soiled linen has been identified as a source of large numbers of certain pathogenic organisms, the risk of actual disease transmission is negligible.

- Soiled linen may be handled as little as possible and with minimum agitation to prevent gross microbial contamination of the air and of persons handling the linen.
- All soiled linen must be handled with gloved hands and if feasible, decontaminated in 0.5- 1% sodium hypochlorite in the laboratory before sending to the laundry.
- Soiled linen after decontamination should be put in heavy plastic bags which are tied and sent to the laundry.
- In the laundry, decontamination in bleach is recommended in case not done earlier. This should be followed by washing in hot water ($>70^{\circ}\text{C}$) with detergent.

1.2.4.7. General biosafety guidelines for laboratory workers

- Eating, drinking, smoking and application of cosmetics are prohibited in the laboratory.
- Sandals and open style shoes do not afford proper foot protection and are not to be used.
- As far as possible lenses should not be worn in eyes instead one should wear spectacles.
- Laboratory and work tables should be scrupulously cleaned with liquid detergents and disinfectants. Laboratory work surface should be decontaminated once a day after completion of day's activity and immediately after spill of viable material with disinfectant.
- Centrifuge safety caps should be used whenever handling hazardous specimens and when it is likely to produce aerosols or infectious droplets.
- Blood and other specimen containers should be labeled with a warning sign. The outside of the specimen container should be cleaned with sodium hypochlorite solution in case of visible contamination.
- Gloves should be worn while dealing with blood specimens, blood-soiled items, body fluids, excretions, secretions, surface materials and objects exposed to them.
- Hands should be washed immediately after contact with blood and before leaving the laboratory.
- Hands should always be washed before wearing and after removing gloves.
- Gowns / laboratory coats must be worn while working with potentially infective materials and removed before leaving the laboratory.
- **Recapping or bending of needles is strictly prohibited.** Needles should be destroyed with needle destroyer and remaining hub should be put in puncture proof container.
- Syringes should be put in freshly prepared bleach solution.
- Paper work should not be done on potentially contaminated surface.
- All potentially contaminated materials and wastes from the laboratory should be disposed after decontamination preferably by autoclaving / incineration.

1.2.4.8.. Bio safety Management

- Designate one person responsible for bio-safety activities, e.g. DMS/ Microbiologist/ MO CHC/PHC or senior laboratory technician.
- Appropriate medical evaluation, surveillance and treatment should be provided for all personnel in case of need, and adequate medical records should be maintained.
- Immunization against diseases which are feasible must be given according to the schedule, especially against Hepatitis B etc.
- Staff should receive regular training in laboratory bio-safety and should be updated at regular interval. A copy of the Bio-safety manual should be available in the laboratory at all times.

1.2.4.9. Training

Human error and poor techniques are important in non protection of laboratory workers. Continuous in-service training in safety measures is essential for health care workers to minimize human errors and improve laboratory techniques.. An effective safety program begins with the laboratory in charge, which should ensure that safe laboratory practices and procedures are being followed. Employees should be introduced to the code of **GMT** and to the Bio-safety manual. Staff training should include safe methods adopted for commonly used laboratory procedures like:

- Inhalation risks: using loops, streaking agar plate, pipetting, smear preparation, opening culture stocks, centrifugation, taking blood/serum samples etc.
- Ingestion risks: handling specimens, smears, cultures.
- Inoculation risks: accidental needle stick injuries.
- Handling blood and other infectious agents.
- Decontamination and disposal of infectious material.

1.2.4.10. Health and medical check up

The Microbiologist /Pathologist at District Public health laboratory could help in ensuring that there is regular health check up of laboratory personnel. The objective of such check up is to monitor for occupationally acquired diseases.

- Provision of immunization:

It is recommended that all laboratory persons receive protective immunization against the disease they are dealing with specially **Hepatitis B** which plays a key role in prevention of transmission of HBV from patient to health care worker. Therefore it is important that all the HCW's including the laboratory workers should be immunized with 3 doses of hepatitis B as per approved schedule (0, 1 and 6 months interval)Health care worker should receive booster dose after 5-6 years interval.

- Regular health checkup of the staff for diseases including collection of samples where ever indicated.
- The record for the same should be maintained.

1.2.4.11.Waste management

Waste is anything that is to be discarded. In laboratories, decontamination of wastes and their ultimate disposal are closely interrelated. The overriding principle is that all infectious materials should be decontaminated, autoclaved or incinerated before disposal. The principal questions to be asked before discharge of any objects or materials from laboratories that deal with potentially infectious microorganisms are:

- Have the objects or materials been effectively decontaminated or disinfected by an approved procedure?
- If not, have they been packaged in an approved manner for immediate on-site incineration or transfer to another facility with incineration capability?
- Does the disposal of the decontaminated objects or materials involve any additional potential hazards, biological or otherwise, to those who carry out the immediate disposal procedures or who might come into contact with discarded items outside the facility?
- Biomedical waste management is given in detail in chapter5.

Chapter 2

Laboratory Equipment-Safe Operations and maintenance

While proper and safe use of laboratory equipment is essential to ensure safety in microbiology laboratories, this equipment also requires proper operation and maintenance. This section deals with use and maintenance of some important bio-safety equipment provided for public health laboratories under IDSP.

There are different types of microbes and they have been classified into 4 risk groups.

Microbial risk assessment

This risk group classification is to be used for laboratory work only. Table 1 describes the risk groups.

Table 1. Classification of infective microorganisms by risk group

RISK GROUP	DEFINITION	EXAMPLE
Risk Group 1 (no or low individual and community risk)	A microorganism that is unlikely to cause human or animal disease	<ul style="list-style-type: none">▪ <i>Bacillus subtilis</i>▪ <i>Lactobacillus spp</i>▪ <i>Staphylococcus</i>▪ <i>E. coli</i>
Risk Group 2 (moderate individual risk, low community risk)	A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited	<ul style="list-style-type: none">▪ <i>Measles virus</i>▪ <i>Salmonella sp</i>▪ <i>Toxoplasma sp.</i>▪ <i>Hepatitis B virus</i>▪ <i>HIV (non culture based)</i>
Risk Group 3 (high individual risk, low community risk)	A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.	<ul style="list-style-type: none">▪ <i>Hanta virus</i>▪ <i>Y pestis</i>▪ <i>Avian Flu</i>▪ <i>Bacillus anthracis</i>▪ <i>HIV culture</i>
Risk Group 4 (high individual and community risk)	A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.	<ul style="list-style-type: none">▪ <i>Ebola virus</i>▪ <i>Variola virus</i>▪ <i>Nipah virus</i>▪ <i>Marburg virus</i>

Organism depending on their virulence and severity of infection it causes they are classified as follows.

Biological safety cabinets

Table 2.

Summary of recommended BSLs for infectious agents

BSL	Agents	Practices and techniques	Safety equipment	Facilities
1	No disease in healthy adults (e.g., <i>B subtilis</i>)	Standard microbiological practices	None required Laboratory clothing recommended Protection of skin lesions and eyes if indicated	Open bench top resistant and impervious to water Sink required
2	Associated with human disease Transmission generally not via aerosols (e.g., <i>Salmonella spp</i>)	BSL 1 plus: * Limited access * Biohazard label * Sharp object precautions * Bio-safety manual (including waste decontamination immunization policies, training).	Class I or II BSC or other containment devices if infectious aerosols or splashes may occur Appropriate PPE	BSL-1 plus: * Autoclave * Eyewash facility
3	Serious or lethal consequences Potential for aerosol transmission (e.g., <i>M tuberculosis</i>)	BSL-2 plus; * Controlled access * Decontamination of all waste and clothing * Baseline serum sample	Class I or II BSC or other containment devices for all procedures, Appropriate PPE	BSL-II plus: * Negative air flow * Air Exhaust to outside * Self closing, double doors
4	Life threatening Transmission by aerosol or unknown risk of transmission (e.g. <i>Ebola virus</i>)	BSL-3 plus: * Facility specific clothing * Shower on exit * Decontamination of all materials on exit	Class III or class II BSC in combination with full body, air supplied, positive-pressure suit for all procedures	BSL- 3 plus separate building and special engineering and design features

2.1 Biological safety cabinets (BSCs) are designed to protect the operator, the laboratory environment and work materials from exposure to infectious aerosols and splashes that may be generated when manipulating materials containing infectious agents, such as primary cultures, stocks and diagnostic specimens

Aerosol particles are created by any activity that imparts energy into a liquid or semi liquid material, such as shaking, pouring, stirring or dropping liquid onto a surface or into another liquid. . BSCs are highly effective in reducing laboratory-acquired infections and cross-contaminations of cultures due to aerosol exposures & also protect the environment.

An essential component of the BSCs is the high-efficiency particulate air (HEPA) filter in the exhaust system. The HEPA filter traps 99.97% of particles of 0.3 μm in diameter and 99.99% of particles of greater or smaller size. This enables the HEPA filter to effectively trap all known infectious agents and ensure that only microbe-free exhaust air is discharged from the cabinet. These basic design concepts have led to the evolution of three classes of BSCs. Class I and Class II has been explained here.

2.1.1. Class I biological safety cabinet

Room air is drawn in through the front opening (for use by the operator's arms to reach the work surface inside the cabinet), it passes over the work surface and is discharged from the cabinet through the exhaust duct. The directional flow of air whisks aerosol particles that may be generated on the work surface away from the laboratory worker and into the exhaust duct. The air from the cabinet is exhausted through a **HEPA filter** to the outside. It provides personnel and environmental protection but because un-sterilized room air drawn over the work surface through the front opening, does provide consistently unreliable product protection.(fig 11)

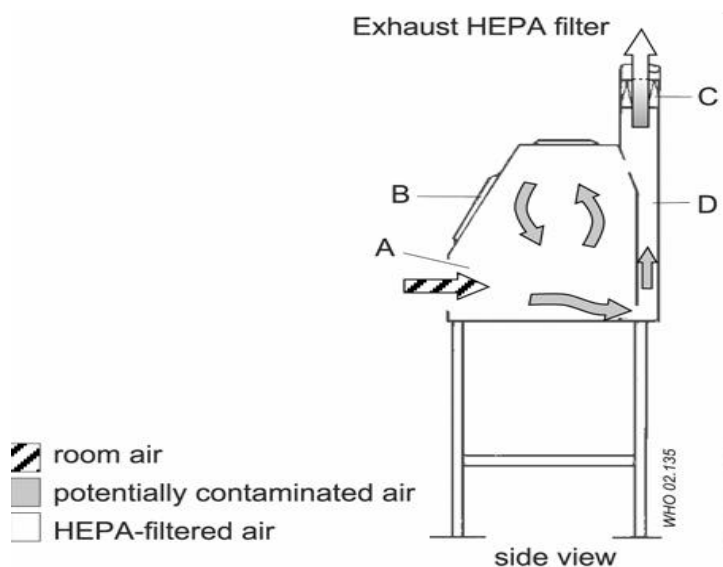


Fig: 11 Schematic diagram of a Class I biological safety cabinet. A: front opening, B: sash, C: exhaust HEPA filter, D: exhaust plenum.

2.1.2. Class II biological safety cabinets

The Class II BSC is designed not only to provide personnel protection but also to protect work surface materials from contaminated room air. (Fig 12) **Class II BSCs**, differ from **Class I BSCs** by allowing only air from a **HEPA-filtered** (sterile) supply to flow over the work surface. The Class II BSC can be used for working with infectious agents in Risk Groups 2 and 3.

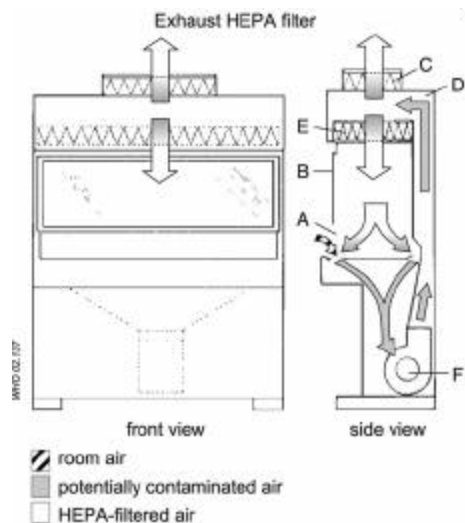


Fig:12 Schematic representation of a Class IIA1 biological safety cabinet. A: front opening, B: sash, C: exhaust HEPA filter, D: rear plenum, E: supply HEPA filter, F: blower.

2.2 General operations and maintenance instructions:

2.2.1. Using biological safety cabinets in the laboratory Location

- BSCs should be situated in a location away from common movement of laboratory workers and potentially disturbing air currents.
- Whenever possible, a 30-cm clearance should be provided behind and on each side of the cabinet to allow easy access for maintenance.
- A clearance of 30–35 cm above the cabinet may be required to provide for accurate air velocity measurement across the exhaust filter and for exhaust filter changes.

2.2.2. Operator

- The frequent in-and-out movement needed to use these containers is disruptive to the integrity of the cabinet's air barrier, and can compromise both personnel and product protection. Therefore, number of movements across the front opening should also be minimized by placing all necessary items into the cabinet before beginning manipulations.

2.3. Personal protective equipment while using BSC

- Personal protective clothing should be worn whenever using a BSC.
- Laboratory coats are acceptable for work being performed at Bio-safety Levels 1 and 2.
- Gloves should be pulled over the wrists of the gown rather than worn inside.
- Masks and safety glasses may be required for some procedures.

2.4. Material placement

- The front intake grill of Class II BSCs must not be blocked with paper, equipment or other items.
- Materials to be placed inside the cabinet should be surface-decontaminated.
- All materials should be placed as far back in the cabinet, towards the rear edge of the work surface, as practical without blocking the rear grill.
- Aerosol-generating equipment (e.g. mixers, centrifuges, etc.) should be placed towards the rear of the cabinet.
- Active work should flow from clean to contaminated areas across the work surface.

2.5. Disposable transfer loops

- Do not have to be sterilized and therefore be used in biological safety cabinets where Bunsen burners would disturb the airflow.
- These loops should be placed in disinfectant after use and discarded as contaminated waste

2.6. Operation and maintenance

- Cabinets should be turned on at least 5 min before beginning work and after completion of work to allow the cabinet to “purge”, i.e. to allow time for contaminated air to be removed from the cabinet environment.

2.7. Ultraviolet lights

- Ultraviolet lights are not required in Bio-safety cabinets.
- However, if one is using it then it must be cleaned weekly/ regularly to remove the dust.
- Ultraviolet lights intensity should be checked on regular basis to ensure the light intensity is proper.
- Protect your eyes & skin from exposure to ultraviolet rays whenever light is on.

2.8. Open flames

- Open flames should be avoided in the near microbe-free environment created inside the BSC. They disrupt the airflow patterns and can be dangerous when volatile, flammable substances are also used.

2.9. Spills

- When a spill of bio-hazardous material occurs within a BSC, clean-up should begin immediately, while **the cabinet continues to operate**.
- An effective disinfectant (1% sodium hypochlorite) should be used and applied in a manner that minimizes the generation of aerosols.
- All materials that come into contact with the spilled agent should be disinfected and/or autoclaved.

2.10. Cleaning and disinfection

- All items within **BSCs**, including equipment, should be surface-decontaminated and removed from the cabinet when work is completed, since residual culture media may provide an opportunity for microbial growth.
- The interior surfaces of **BSCs** should be decontaminated before and after each use. The work surfaces and interior walls should be wiped with a disinfectant that will kill any microorganisms that might be found inside the cabinet.
- At the end of the work day, the final surface decontamination should include a wipe-down of the work surface, the sides, back and interior of the glass. A solution of bleach or 70% alcohol should be used where effective for target organisms. A second wiping with sterile water is needed when a corrosive disinfectant, such as bleach, is used.
- The cabinet should be kept running for at least 5 min in order to purge the atmosphere inside before it is switched off.

2.11. Decontamination

- BSCs must be decontaminated before filter changes and before being moved. **The most common decontamination method is by fumigation with formaldehyde gas (see box below).**

2.12. Annual Certification

Evaluation of the Bio-safety cabinets should include tests for cabinet integrity, HEPA filter leaks, down flow air flow velocity etc. In order to maintain the equipment in view of this Annual Maintenance Contract may be done and certificate be obtained by the firm.

Decontamination of biological safety cabinets

- For decontamination of Class I and Class II cabinets, the appropriate amount of Para-formaldehyde (final concentration of 0.8% Para-formaldehyde in air) should be placed in a frying pan on an electric hot plate.
- Another frying pan, containing 10% ammonium bicarbonate than Para-formaldehyde, on a second hot plate is also placed inside the cabinet.
- The hot plate leads are plugged in outside the cabinet, so that operation of the pans can be controlled from the outside by plugging and unplugging the hot plates as necessary.
- If the relative humidity is below 70%, an open container of hot water should also be placed inside the cabinet before the front closure is sealed in place with strong tape (e.g. duct tape).
- Heavy gauge plastic sheeting is taped over the front opening and exhaust port to make sure that the gas cannot seep into the room.
- The plate for the Para-formaldehyde pan is plugged in.
- It is unplugged when all the Para-formaldehyde has vaporized.
- The cabinet is left undisturbed for at least 6 h.
- The plate for the second pan is then plugged in and the ammonium bicarbonate is allowed to vaporize.
- This plate is then unplugged and the cabinet blower is switched on for two intervals of approximately 2 Seconds each to allow the ammonium bicarbonate gas to circulate.
- The cabinet should be left undisturbed for 30 min before the front closure (or plastic sheeting) and the exhaust port sheeting are removed.
- The cabinet surfaces should be wiped down to remove residues before use.

Chapter3

Good Microbiological Techniques-Safe laboratory procedures

Human error, poor laboratory techniques and misuse of equipment cause the majority of laboratory injuries and work-related infections. This chapter provides a short note on technical methods that are designed to avoid or minimize the most commonly reported problems of this nature.

3.1. Safe handling of specimens in the laboratory

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

3.1.1 Specimen containers

- ✓ Specimen containers may be of glass or preferably plastic.
- ✓ They should be robust and should not leak when the cap or stopper is correctly applied. A specimen container without cap/stopper is simply unacceptable for Sample collection.
- ✓ No material should remain on the outside of the container.
- ✓ Containers should be correctly labeled to facilitate identification. Figure 13 shows an example of sample label
- ✓ Do not put samples on request forms.(Fig 14)
- ✓ Specimen request or specification forms should not be wrapped around the containers but placed in separate, preferably waterproof envelopes .Whenever the specimen needs to be transported.

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

Fig 13: LABELLING AND IDENTIFICATION OF SPECIMENS



Fig 14: Do not keep samples on request forms.

3.1.2 Transport of specimens within the facility

- ✓ To **avoid accidental leakage or spillage**, secondary containers, such as boxes, should be used, fitted with racks so that the specimen containers remain upright.
- ✓ The secondary containers may be of metal or plastic, should be autoclavable or resistant to the action of chemical disinfectants, and the seal should preferably have a gasket. They should be regularly decontaminated.

3.1.3 Receipt of specimens

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

3.1.4 Opening packages

- ✓ Personnel **who receive and unpack specimens** should be aware of the potential health hazards involved, and should be trained to adopt standard precautions, particularly when dealing with broken or leaking containers.
- ✓ Primary specimen containers should be opened in a biological safety cabinet.
- ✓ Disinfectants should be available.

3.2 Separation of serum

- Always follow standard operative procedure and take care of following
- Hands, eye and mucous membrane protection should be worn.(Universal precaution)
- Splashes and aerosols can only be avoided or minimized by good laboratory techniques. Blood and serum should be pipetted carefully, not poured. Pipetting by mouth must be forbidden.
- After use, pipettes should be completely submerged in suitable disinfectant. They should remain in the disinfectant for the appropriate time before disposal or washing and sterilization for reuse(15 min).
- Discarded specimen tubes containing blood clots, etc. (with caps replaced) should be placed in suitable leak-proof containers for autoclaving and/ or incineration.
- Suitable disinfectants should be available for clean-up of splashes and spillages

3.3 Use of centrifuges

- Satisfactory mechanical performance is a prerequisite of microbiological safety in the use of laboratory centrifuges.
- Centrifuges should be operated according to the manufacturer's instructions.
- Centrifuges should be placed at such a level that workers can see into the bowl to place trunnions and buckets correctly.
- **Tubes and specimen containers should always be securely capped (screw-capped if possible) for centrifugation.**
- Buckets and trunnions should be paired by weight and, with tubes in place, correctly balanced.
- The amount of space that should be left between the level of the fluid and the rim of the centrifuge tube should be given in manufacturer's instructions to be followed.
- Distilled water or alcohol 70% should be used for balancing empty buckets. Saline or hypochlorite solutions should not be used as they corrode metals.
- The interior of the centrifuge bowl should be inspected daily for staining or soiling at the level of the rotor. If staining or soiling is evident then the centrifugation protocols should be re-evaluated.
- Centrifuge rotors and buckets should be inspected daily for signs of corrosion and for hair-line cracks.
- Buckets, rotors and centrifuge bowls should be decontaminated after each use.
- After use, buckets should be stored in an inverted position to drain the balancing fluid.
- Infectious airborne particles may be ejected when centrifuges are used. These particles travel at speeds too high to be retained by the cabinet airflow if the centrifuge is placed in a traditional open-fronted Class I or Class II biological safety cabinet. However, good centrifuge technique and securely capped tubes offer adequate protection against infectious aerosols and dispersed particles.

3.4 Glass and "sharps"

- Plastics should replace glass wherever possible. Only laboratory grade (borosilicate) glass should be used, and any article that is chipped or cracked should be discarded.
- Hypodermic needles must not be used as pipettes
- Before discarding disposable syringes/needles, mutilate/ disfigure to avoid reuse or picking up by rag pickers for recirculation.

3.5 Films and smears for microscopy

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

3.6 Opening of ampoules containing lyophilized infectious materials

Care should be taken when ampoules of freeze-dried materials are opened, as the contents may be under reduced pressure and the sudden inrush of air may disperse some of the materials into the atmosphere. **Ampoules should always be opened in a biological safety cabinet.** The following procedures are recommended for opening ampoules.

- First decontaminate the outer surface of the ampoule.
- Make a file mark on the tube near to the middle of the cotton or cellulose plug, if present.
- Hold the ampoule in alcohol-soaked cotton to protect hands before breaking it at a file scratch.
- Remove the top gently and treat as contaminated material.
- If the plug is still above the contents of the ampoule, remove it with sterile forceps.
- Add liquid for re suspension slowly to the ampoule to avoid frothing.

3.7 Decontamination

- Hypochlorite and high-level disinfectants are recommended for decontamination.
- Freshly prepared hypochlorite solutions should contain available chlorine at 1 g/l for general use and 5 g/l for blood spillages.
- It should be prepared daily and at end of the day it should be discarded.

CHAPTER -4

Sterilization and Disinfection Procedures

Definition

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

Disinfection is a process which reduces the number of pathogenic micro-organisms, but not necessarily bacterial spores from inanimate objects or skin.

Disinfectants used should be in proper concentration and for suitable period of time which will be effective against all the organism.

High level disinfection is often used for a process which kills *Mycobacterium tuberculosis* and Enteroviruses in addition to other vegetative bacteria, fungi and more sensitive viruses.

Sterilization is a process which destroys all micro-organisms including bacterial spores. The level of decontamination should be such that there is no risk for infection when using the equipment.

4.1 Classification of infection risk from equipment or environment into three categories and suggested levels of decontamination

Low risk: Items in contact with normal and intact skin, or the inanimate environment not in contact with the patient (e.g. walls, floors, ceilings, furniture, sinks and drains). Cleaning and drying is usually adequate except when there is spill of blood/ body fluids.

Intermediate risk: Equipment which does not penetrate the skin or enter sterile areas of the body but is in contact with mucous membranes or non-intact skin, or other items contaminated with virulent or transmissible organisms (e.g. respiratory equipment, gastrointestinal endoscopes, vaginal instruments, thermometers). High level disinfection is usually adequate.

High risk: Items penetrating sterile tissues, including body cavities and the vascular system (e.g. surgical instruments, intra-uterine devices, vascular catheters, syringes and needles etc.). Decontamination followed by cleaning and sterilization is required. High level disinfection may sometimes be appropriate if sterilization is not possible.

4.2: Environmental cleaning:

- Floors, surfaces, sinks and drains should be cleaned with warm water and detergent. Routine use of disinfectants is unnecessary.
- If there is spillage of blood, body fluids or sputum, disinfection before cleaning is recommended. In high risk areas or following spillage from a known infected patient, the surface is cleaned using freshly prepared 0.5-1% sodium hypochlorite solution. Gloves should be worn. Thorough cleaning should be done.

4.3 Hand decontamination

- Good hand washing is the most important step in preventing disease transmission in health settings.
- Alcoholic hand rubs are not a substitute for hand washing, except for rapid hand decontamination between patient contacts.
- The specific hand disinfectants - antiseptics recommended are: 2-4% chlorhexidine, 5-7.5% povidone
- Iodine, 1 % triclosan or alcoholic rubs.

4.4 Decontamination/disinfection of used needles and syringes:

- The needle is not detached from syringe.
- Do not recap the needle.
- The disinfectant is aspirated into the syringe.
- The needles and syringes are immersed with disinfectant horizontally in a flat tray for 30 minutes .
- The disinfectant solution is discharged from the syringe and needle
- The disposable syringes and needles are disposed.
- The reusable syringes and needles are autoclaved/ boiled for 30 minutes.

4.5. Disinfection of disposable items

Material required

- 1% sodium hypochlorite / 3% Lysol solution.
- Glass jar.
- Bio-safety bag (puncture resistant with appropriate color code).
- Gloves.

Procedure

- Freshly prepare requisite quantity of disinfectant in a jar meant for this purpose.
- Put articles to be discarded in the solution overnight.
- Drain off disinfectant.
- Collect the material in safety bags & dispose off along with other garbage at
- Designated place.

GLP (Good Laboratory Practices)

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

Table 4.1 Common Disinfectants and their use:

Disinfectant	Articles	Comments
Sodium hypochlorite 1% To prepare 1% dilution - 5% solution to be diluted 1:5 in tap water.	Disinfection of material contaminated with blood and body fluids	<ul style="list-style-type: none">• Should be used in well-ventilated areas.• Protective clothing required while handling and using undiluted• Not to be mixed with strong acids to avoid release of chlorine gas• Corrosive to metals
Bleaching powder 7g/litre with 70% available chlorine .may be used in place of liquid bleach if liquid bleach is unavailable	Toilets, bathrooms.	Same as above
Alcohol (70%) Isopropyl, ethyl alcohol, methylated spirit.	Smooth metal surfaces, table tops and other surfaces on which bleach cannot be used.	<ul style="list-style-type: none">• Flammable, toxic, to be used in well-ventilated area, avoid inhalation.• To be kept away from heat sources, electrical equipment, flames, hot surfaces.• Should be allowed to dry completely particularly when

Glutaraldehyde (2%)	For disinfection of endoscopes, respiratory therapy equipment and for materials that are destroyed by heat. Can work as a sterilant if contact time is 6-8 hrs and if used under strictly controlled condition	<ul style="list-style-type: none"> • Eye and nasal irritant, may cause asthma and skin allergies, hence should be used in well ventilated area, keep covered with well fitting lids. • Eye protection, plastic apron and gloves should be worn while handling
Detergent with enzyme	Cleaning endoscopes, surgical instruments before use	
Chlorhexidine combined with alcohol or detergents	Antiseptic, for skin and mucous membranes, preoperative skin preparation, disinfection of hands	<ul style="list-style-type: none"> • Inactivated by soap, organic matter • Relatively non toxic • Should not be allowed contact with brain meninges/ eye or middle
Quaternary Ammonium Compounds (e.g. Dettol) May be combined with chlorhexidine	Antiseptic, for cleaning dirty wounds (Low level disinfection only)	<ul style="list-style-type: none"> • Relatively non toxic • Dilutions in use are likely to get contaminated and grow gram negative bacteria • Should be used in correct dilution • Solution in use should be Changed every 8 hours • Stock bottle should not be topped up

4.6. Disinfection of reusable articles contaminated with morbid material

Material required

Disinfectant as described above.

Metallic box/ Tray.

Puncture resistant bio-safety bags.

Bunsen burner (Heating device for boiling).

Procedure

After treating the material with suitable solution of disinfectant over night, proceed further as follow.

- Drain off disinfectant in sink fitted with tap.

- Transfer the material in metal box or tray with cover.
- Place on Bunsen burner (heating device) for boiling.
- Wait up to 15-20 min. after the boiling starts.
- Put off the flame & allow cooling the material in metallic box/ tray.
- Drain off water.
- Pass on the material for washing.

4.6.1 .Glassware containing culture material

Material required

Bio-safety puncture resistant bags/ Autoclave.

Procedure

- Discard all the material contaminated with culture material directly into metal box / puncture resistant bio-safety bags. Place box/ bio-safety bags with material to be decontaminated in autoclave designated for this work only. Decontaminate the material by autoclaving.. Drain off culture media and pass on material for further washing etc.

GLP

Be sure that decontamination should only be done with autoclaves designated for this purpose. Autoclave should be checked for its efficacy using chemical indicator (**Fig 15**)



Fig 15: Indicator tape Indicator tape

4.7. Washing of laboratory glassware

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

4.7.1. New glassware

Purpose

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

Material required

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

Procedure

- Prepare sufficient quantity of 2 % hydrochloric acid (e.g. 98 ml of water & 2.0 ml hydrochloric acid) as per the requirement in a big plastic basin.
- Wash the newly received glassware under running tap water to remove the visible dust sticking inside and/or out side surface of the article.
- Soak the already washed articles in 2% hydrochloric acid solution.
- Leave them there overnight.
- Take the articles from 2 % hydrochloric acid and rinse in clean water twice.
- Finally wash using demineralized water. Allow to dry using hot air oven.
- Pass on for packing & sterilization for further use.

GLP

Care should be taken while using HCl.

Add acid to water drop by drop by constant stirring (and not vice versa)

4.7.2. Dirty glassware

Material required

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

Procedure

- Take material, glassware etc. already decontaminated (chemically/ autoclaving) and rinse twice in Luke warm water to remove any dirty stain sticking on them
- Put the material to be washed in bowl containing 1% detergent solution.
- Allow to boil (Electrically / by Bunsen burner flame).

- While in solution, scrub inside & outside surface of the glassware with the help of the brush.
- Leave the glassware in the solution for 2 - 3 hrs.
- Take out each article one by one and rinse under running tap water till no trace of detergent is left, which otherwise may lead to false results when used.
- Drain the water by putting each article on a wall draining rack or by keeping articles upside down in a wire basket.
- Put articles in wire basket and keep in hot air oven at 60°C for drying purpose only.
- Take out each article and plug using non-absorbent cotton/aluminum foil.
- Pass on for sterilization (dry heat/ autoclaving)
- In case of delay, store in dust free area.

4.8. METHOD OF STERILIZATION

4.8.1 Sterilization:

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

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

- Dry heat,
- Moist heat.
- Filtration.

4.8.2 Dry heat

The commonly used methods to sterilize the material are as follows:

Red heat flaming

Hot air sterilization.

4.8.3 Red heat flaming

Purpose

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

Material required

Bunsen burner attached to gas supplies. Match box.

Procedure

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

GLP

Each time when heating in the Bunsen burner flame, allow to cool down the instrument. Check loop/ wire etc. by touching a portion of the medium to be inoculated. Heat the loop vertically so that the entire length of the loop is heated. Dip the loop in disinfectant solution before heating to avoid splattering.

4.9 Hot air Sterilization

Purpose

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

Equipment required

Hot air oven. (Fig 16)



Fig 16: Hot air oven

Procedure

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

Temperature Holding Time

160 °C for 60 minutes.

170 °C for 40 minutes.

180 °C for 30 minutes.

The most common temperature for hot air oven for sterilization is 160 °C for 60 min. On expiry of the holding time period, switch off the power supply and allow the oven to cool down slowly. Put down the date of sterilization on each packet and store in dust free area for future use. Make daily records of the equipment/ material sterilized as per the Performa given below.

Date	Detail of Items to be Sterilized	Pressure at which sterilization done	Starting time		Heating time		Holding time	Chemical indicator tape (color changed)
			From	To	From	To		

GLP

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

4.10 Moist heat

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

Below 100°C

Pasteurization 63°C - 80°C for 30minutes.

Tyndaliation: Intermittent exposure at 75 - 80°C for 20 - 45 minutes on three successive days.

Boiling at 100°C for 5 - 10 minutes.

Steaming at 100°C for 1 hr.

Steaming under pressure (Autoclaving)

Purpose

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

Principle

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



Fig 17: Autoclave

Item to be sterilized

Autoclave (Fig 17) is mostly suitable for:

- Sterilization of culture media, aqueous solution.
- Decontamination of discarded culture and other laboratory garbage.
- Rubber guard, gloves, stoppers with rubber liner, glassware with rubber attachment, glass metal syringes, throat swabs etc.

Type of Autoclaves

In principle two type of autoclaves are used

- Pressure cooker type.
- Gravity displacement type.

4.11.1 Pressure cooker type (Fig 18)

This is the most common type of autoclave used for sterilization. It has vertical chamber with a strong metal lid, which can be fastened down, and sealed with rubber gasket. An air steam discharger tap, pressure gauge and safety cum pressure adjustable valve are fitted on the lid. Water in the bottom of the autoclaves is heated electrically (or by some other device like gas burner/ kerosene oil).



Fig 18: Pressure cooker with stove

Temp Pressure Time

115°C 10 lbs/ inch. 20 - 30 min.

121°C 15 lbs/ inch 15- 20 min.

132°C 27 lbs/ inch 2 min.

- Holding time increases to 30-45min if at 121°C and 15 lbs pressure if plastic wares are sterilized
- At the end of holding time switch off the power supply.
- Allow the autoclave to cool slowly which can be seen by gradual decrease in pressure till it shows zero reading.
- Allow the wrapping paper to be dried.
- Put date on each article and place in dust free area for future use.

GLP

- Ensure that air from chamber has been expelled completely because air steam mixture has a lower temperature than steam e.g. temperature of 50% air & 50% steam mixture will be 112°C instead of 121°C provided by the pure steam.
- Air also hinders the penetration of steam in to the interior of the porous material and narrow opening container. Air being denser than steam tends to form a separate cooler layer in the bottom of the autoclave.
- As the simple autoclave lack means for drying the load after sterilization, it is therefore important to avoid placing sterilized articles in contact with unsterilized objects/ surface unless the wrapping is dried
- To check the efficacy of autoclave, each cycle should be run using chemical indicator tape.

Record Keeping

Daily recording of each run for sterilization of material should be maintained

Date Detail of Pressure and Holding time

4.10.2 Gravity displacement type

Autoclaves with air discharge by gravity displacement

these autoclaves are usually arranged horizontally and are rectangular in shape, thus making the chamber more convenient for loading. The door should have a safety device to ensure that it cannot be opened while the chamber is under pressure.

4.11 Quality control

To check the efficacy of autoclave each run should be accompanied by placing chemical indicator which changes color if the instruments is working satisfactory. This can be achieved by placing chemical indicator tape inside the tube in the center of autoclave and check the change in color after the operation is over.

Chapter 5

Biomedical waste management

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

5.1.1. Safe disposal of laboratory wastes

Laboratory wastes are potential hazards. Infectious waste can transmit numerous diseases in the community and also those who handle waste and live on its proximity, are at risk. Besides, the increasing use of disposables in health care is also posing an additional burden on the waste management facility. It is extremely important that the recycling of these items is prevented. Only a small percentage (<10%) of the waste generated in health care settings are infectious while another 5% is non-infectious but hazardous. **The most practical approach to the management of biomedical waste is to identify and segregate infectious waste (with a potential for causing infection during handling and disposal), for which some special precautions appear prudent.** This will drastically reduce the cost of the disposal methods in health care settings.

5.1.2. Setting up of biomedical waste facility

Every hospital, nursing home, veterinary institution, animal-house, blood banks, research institutes generating biomedical wastes should install an appropriate biomedical waste facility in the premises or should set up a common facility in accordance with the directions given by the appropriate authority. Biomedical waste should not be generated without authorization. Every hospital should have a waste management programme. Waste survey is an important part of the waste management programme and helps in determining both the type and quantity of waste being generated in the hospital including the laboratory and determine the feasible methods of disposal.

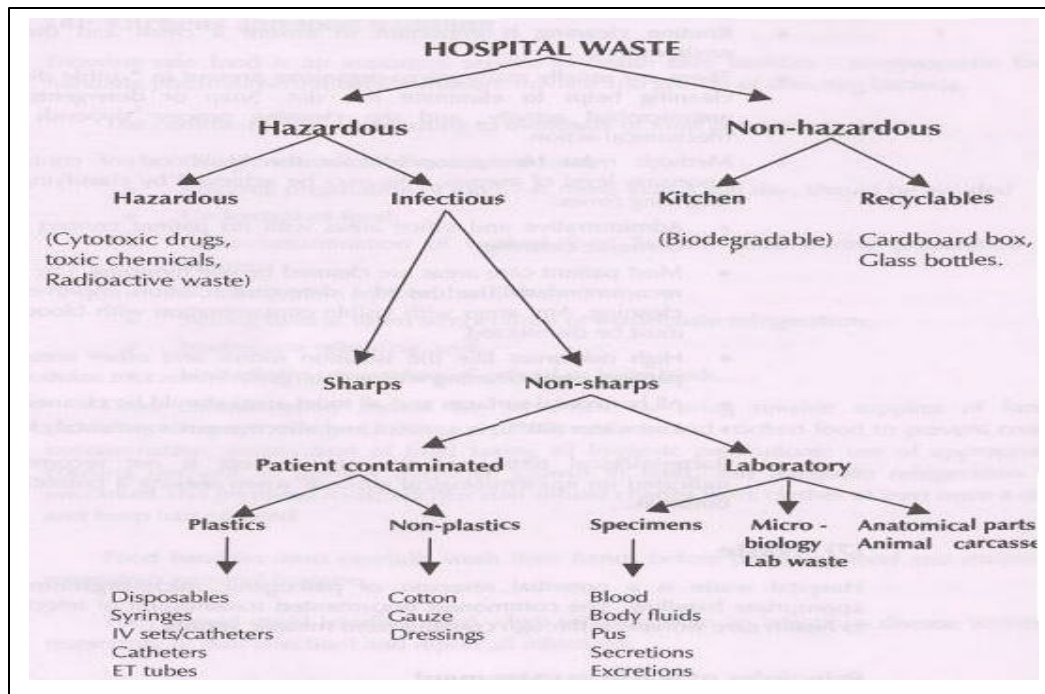
5.2. Principles of waste management

The "Cradle to grave" concept of waste management

- Segregation of wastes into the prescribed categories must be done at the source i.e. at the point of generation.
- Hospital/ laboratory waste requires management at every step from generation, segregation, collection, transportation, storage, and treatment to final disposal.

- Color coded bags as per national/ international norms need to be placed in appropriate containers with the appropriate label/logo e.g. biohazard symbol for infectious waste.
- Puncture proof containers made of plastic or metal with a biohazard symbol, in blood collection areas, injection trolleys , nursing stations and operation theatres should be made available for collecting metallic wastes.
- A collection system for the transport of segregated wastes i.e. carts need to be provided for transportation of waste to the site of incinerator.
- A storage area for wastes which already has been disinfected prior to incineration needs to be demarcated.

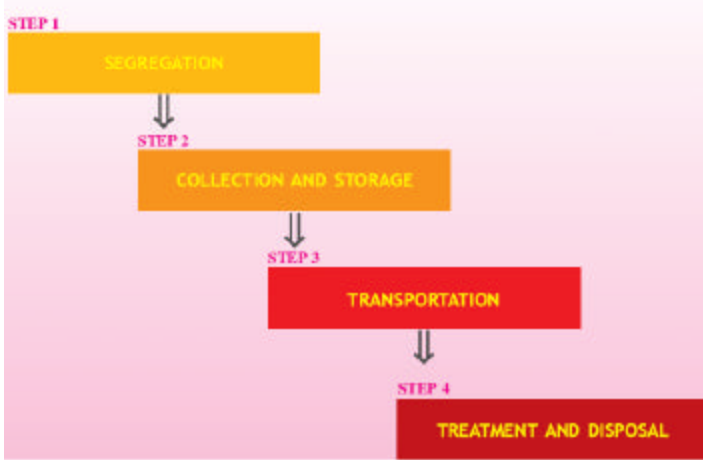
5.3. Practical Classification of Hospital Waste



5.4 .Collecting waste at generation point:

At the generation point ie. The laboratory, waste is managed in the following way

1. Steps For Waste Management



5.4.1Waste segregation

It is the key to any waste management scheme. It consists of placing different types of waste in different containers or colour-coded-bags at the site of generation. This helps in reducing the bulk of infectious waste and contains spread of infection to general waste. This practice reduces the total treatment cost, the impact of waste in the community and the risk of infecting workers. Proper segregation should identify waste according to source and type of disposal/disinfection.(Table 5.1)

Waste should be segregated into different categories (Table-5.2) at the site of generation ie. In the laboratory and weighed separately at the time the waste is being disposed.

Infectious waste must be separated at the point of generation itself and should be decontaminated prior to its storage, transport and disposal.

Table 5.1

No.	Item To be sterilized	Method of sterilization
1	Solid non-infectious waste	Collect in black bag, dispose as household waste
2	Sharps	Needles and syringe nozzle- shredded in needle-destroyer (if available); if not available decontaminated as in b).
3	Scalpel blades – lancets/broken glass	Put in separate container with bleach, transfer to plastic/ card board box, sealed to prevent spillage, transport to safe pit
4	Glassware	To be disinfected, cleaned and sterilized in hot air oven
5	Culture plates with viable culture	To be autoclaved in plastic autoclavable bags. Media removed, collected in yellow bags and disposed by

		incineration/microwaving/hot air oven. Plates can be reused after sterilization
6	Swabs	To be chemically disinfected followed by incineration
7	Disposable items(such as syringe, gloves, sharps)	Dip in freshly prepared 1 % sodium hypochlorite for 30 min- 1 Hour, disposable items.(Twin bins can be used for this purpose(one inside the other). Do Not reuse these disposable items. Shred, cut or mutilate gloves, syringes before disposal
8	Liquid waste	<p>---Non-infectious chemical waste to be first neutralized with reagents and then flushed in sewer system</p> <p>---Liquid infectious waste to be treated with a chemical disinfectant for decontamination neutralized and flushed into the sewer.</p>

For more detail refer to Annexure II.

5.5 Collection bags

Solid wastes are collected in leak-resistant single heavy duty bags or double bags may be used. Bags having different colour codes (Table 5.3) with red labels mentioning date and details of waste are recommended. The bags are tied tightly after they are three-fourth full.

5.6 Packing, storage and transport

- All segregated and disinfected waste should be packed in proper containers and colour-coded bags with red labels mentioning details of biomedical waste and biohazard signs .All containers used for storage of such waste shall be provided with a properly covered lid.
- Such containers should be inaccessible to scavengers and protected against insects, birds, animals and rain.
- There should not be any spillage during handling and transit of such waste.
- The waste sharps, after pre-treatment should be broken before packing in the container.
- The waste should be transported in vehicles authorized for this purpose only.
- No such waste should be stored in the place where it is generated for a period of more than two days.

5.7 Treatment and disposal

Disposal methods: Disposal may be done by:

- Municipal corporation
- Sanitary landfill

5.7.1 Disposal

- * All contaminated (potentially infectious) materials should be autoclaved in leak-proof containers, e.g. autoclavable, color-coded plastic bags, before disposal.
 - * After autoclaving, the material may be placed in transfer containers for transport to the incinerator.
 - * Reusable transfer containers should be leak proof and have tight-fitting covers. They should be disinfected and cleaned before they are returned to the laboratory for further use.
-
- If incinerator is not available, deep burial in controlled landfill sites is recommended .Decontamination should be carried out before burial.
 - Needles should be disposed off by deep burial after disinfecting with bleach solution.

Incineration (Temp.750⁰C)

Incinerator burns /reduces the infectious waste to ashes and is therefore favored by hospitals. It may be of two types –common or individual. There are some disadvantages like pollution /incomplete melting of needles. Hospitals with more than 30 beds or >1000 patients per month should have an incinerator. Plastics cannot be incinerated.

5.8. Guidelines for waste disposal:

- All biomedical wastes should be treated and disposed of strictly in accordance with the guidelines issued by Ministry of Environment and Forest (CPCB guidelines 1998) see Table 5.1.
- Waste which cannot be incinerated, (plastics) should be pre-treated by disinfection and disposed of in an environmentally sound manner.
- Waste should not be dumped, discharged or disposed in any place other than a site identified for the purpose.
- All treatment and disposal facilities should be located at a specified area away from the general service area of the hospital, public places and residential areas.
- When the treatment option is burial, the pits should be located at sites away from agricultural land, residential areas, ground-and safe water sources. There should be no leakage from the pits into surrounding areas.

- All plastics should be disinfected, shredded and disposed of in an environmentally friendly manner. Recycling of disposables eg. syringes, needles, gloves, transfusion bags etc. should be prevented.
- All liquid waste should be disinfected and flushed in the sinks at the point of generation.
- Biomedical waste should not be disposed off on open land and municipal dustbins. Untreated liquid waste should not be let off into sewers.
- All precautions and personal safety measures should be taken (including provision of protective clothing, masks, gloves, gumboots, goggles, etc. as may be necessary). Hepatitis B vaccine is recommended for affording protection to all personnel engaged in handling biomedical waste, or being exposed to such wastes against infection from handling or exposure.

5.9 Maintenance of records

Separate records for classification of waste and their regular disposal should be maintained in the laboratory. The waste disposal programme should be supervised and monitored regularly.

5.10 Reporting of accidents:

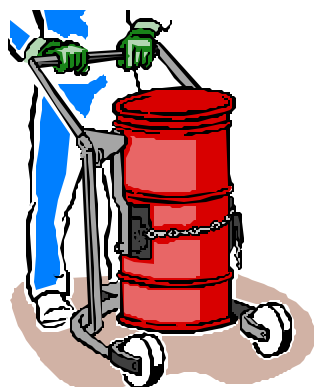
In the event of an accident occurring at any location or site where biomedical wastes are handled or during transportation, the appropriate authorities must be informed and needful action taken



Puncture proof container



Dust bin with colour bags.



Trolleys for carrying waste bags

Fig 19: Steps in waste collection

5.11 Training:

- 5.12** Training regarding need of and national guidelines of **biosafety** practices is extremely important and should be provided at regular intervals for different levels of health care workers. Guidelines for **biosafety** should be provided which may be modified from time to time according to requirement.

Table 5.2

The Ministry of Environment and Forests has a classification, which is notified in the Bio-medical Handling and Management Rules. These have been appended below.

Category	Type of waste	Treatment & Disposal option
Category 1	Human Anatomical wastes (Human tissues, organs ,body parts)	Incineration /deep burial
Category 2	Animal wastes (Animal tissues ,organs, body parts carcasses ,bleeding part ,fluid ,blend and experimental animals used in research waste generated by veterinary	Incineration /deep burial

	hospitals)	
Category 3	<p>Microbiology and Bio-technology wastes</p> <p>(wastes from laboratory cultures ,stocks or specimens of micro-organisms ,live or attenuated vaccines, human and animal cell culture use in research and industrial laboratories wastes from biological productions ,toxins, dishes and devices used to transfer cultures)</p>	Local /Autoclaving /Micro-waving Incineration
Category 4	<p>Waste Sharps</p> <p>(Needles, syringes, scalpels, blades glass etc. that is capable of causing puncture and cuts. This includes both used and unused sharps)</p>	Disinfection(Chemical)/Autoclaving/ Microwaving and mutilation/Shredding
Category 5	<p>Discarded Medicines and Cytotoxic Drugs</p> <p>(Waste comprising of outdated, contaminated and discarded drugs and medicines)</p>	Incineration/Destruction and disposal in land fills
Category 6	<p>Soiled Wastes</p> <p>(Items contaminated with blood and body fluids including cotton ,dressings, soiled plaster ,linens ,bedding other materials contaminated with blood)</p>	Incineration /Autoclaving /Microwaving
Category 7	<p>Solid Wastes</p> <p>(Wastes generated from</p>	Disinfection by chemical treatment / Autoclaving /Microwaving and Mutilation /Shredding

	disposable items other than the waste sharps such as tubing, catheters, IV sets, etc.	
Category 8	Incineration ash (Ash from incineration of any Bio-medical wastes)	Disposal in municipal land fills
Category 9	Chemical Wastes (Chemicals used in biological production, chemicals used in disinfection such as insecticides, etc.)	Chemical treatment and discharge into drains for liquid and secured land fills for solids.

Table 5.3

Colour coding and Containers for Disposal of Biomedical Waste

Waste category	Waste class.	Type of container	Color coding	Treatment of waste disposal
1.	Microbiology & biochem .lab	Plastic holding bags with biohazard sign.	yellow	Autoclaving /incineration
2.	Waste sharps	Reusable plastic/metal containers	blue	deep burial
3.	Discarded chemical, reagents .kits	do	do	do
4.	Soiled wastes (lab coats etc.	Plastic bag with biohazard sign	Yellow	Disinfect/autoclave then machine wash with soap & detergent
5.	Chemical wastes	Sturdy containers or plastic holding bags	Yellow	Incineration(not mercury)

6.	Disposable other than sharps.	Reusable sturdy containers/plastic bags.	blue	Disinfect//shredding/buried.
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REMEMBER

- Do not overfill waste containers
- Fill till $\frac{3}{4}$ full and send for disposal
- Never use hand to force material into overfilled bags
- DO NOT MIX REUSEABLE ITEMS WITH DISPOSABLE ONES.
- Contaminated waste MUST be Autoclaved and NEVER placed in general waste
- If the broken glass is contaminated, it should be treated in the same manner as any contaminated sharps item.
- It is prudent to wipe the top of the Sharps Containers with 10% Bleach solution or other appropriate disinfectant after inserting any contaminated item or at the completion of a task.
- Neither syringes nor needles may be sent to the landfill directly. They must be discarded in "Sharps" Containers only, not in regular trash or autoclaved discard.
- BROKEN GLASS must be Autoclaved and NEVER placed in general waste.
- Do not use cardboard "Broken Glass" boxes.
- Sharps MUST be disinfected and NEVER placed in general waste (Includes ALL disposable syringes, needles, scalpels, etc. and may include pipette tips, glass slides, glass vials. etc.)

Chapter 6

Management of Laboratories accidents

Microbiology laboratories should institute safety precautions according to the potential hazards due to the organisms being handled. A written contingency plan for dealing with laboratory facility accidents is a necessity in any facility that works with high risk organisms

6.1 Essential components of a Contingency plan

The contingency plan should provide operational procedures for:

1. Precautions against natural disasters, e.g. fire, flood, earthquake
2. Biohazard risk assessment
3. Incident-exposure management and decontamination
4. Emergency evacuation of people from the premises

5. Emergency medical treatment of exposed and injured persons
6. Medical surveillance of exposed persons
7. Clinical management of exposed persons
8. Epidemiological investigation
9. Post-incident continuation of operations.

6.2 Emergency procedures for microbiological laboratories

6.2.1 Puncture wounds, cuts and abrasions

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

6.2.2. Needle stick injury

Following steps should be taken

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

Reporting of the Exposure

All percutaneous or mucocutaneous exposures should be reported.

In case of needle stick

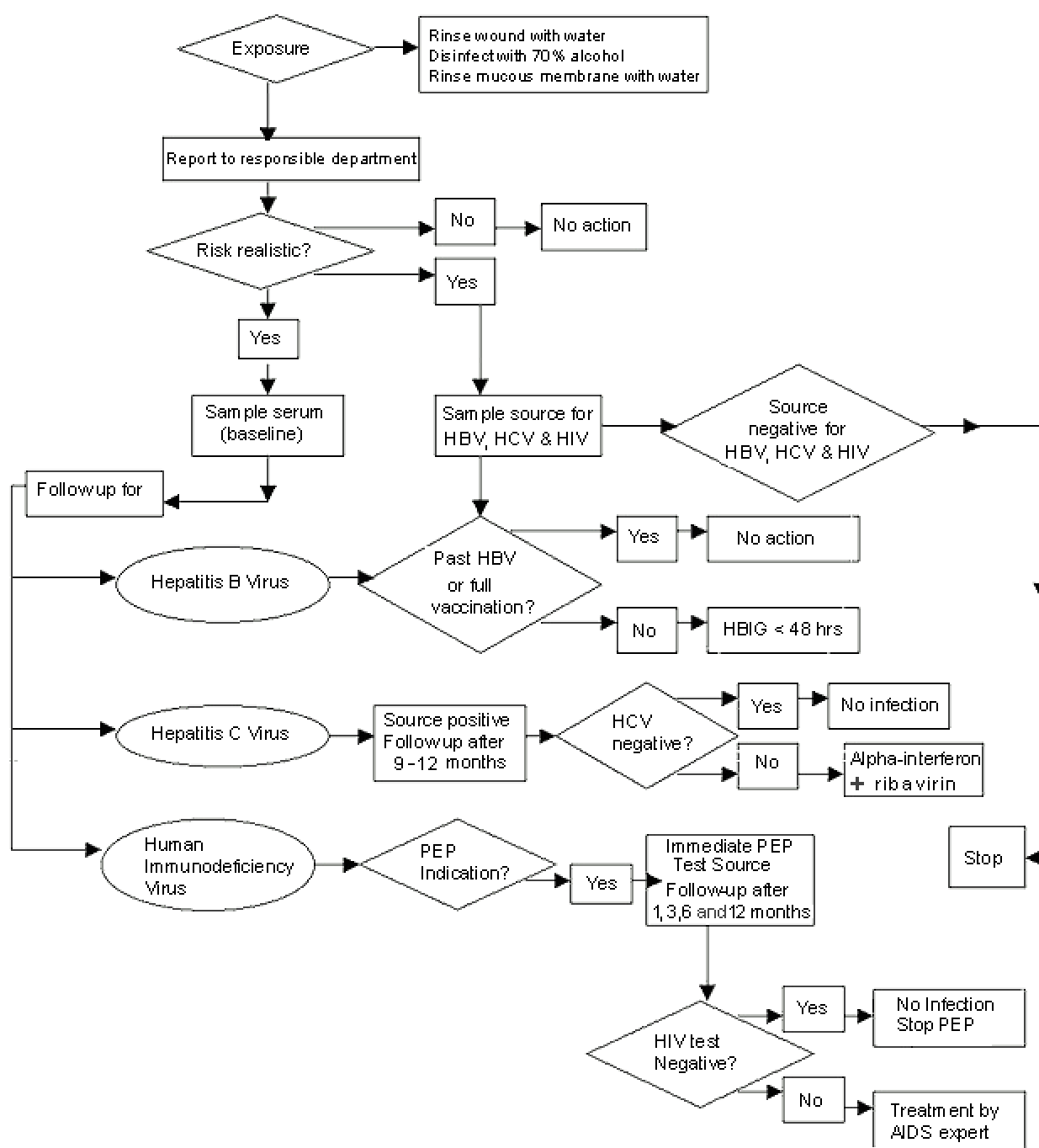
Find out the status of **HIV, HBV and HCV** of the source patient and the health care worker. Ensure confidentiality of laboratory reports so that the HCW is not discriminated.

- In case of HIV negative patient, baseline HIV test of HCW should be done on day of exposure, 6th week and 12th week Simultaneously health worker should receive basic regime of prophylaxis.. .
- In case of HIV positive patient the exposure to the appropriate authority should be informed and condition must be treated as an emergency. Prompt reporting is essential because in some cases, HIV **post exposure prophylaxis (PEP)** may be recommended and it should be started as soon as possible preferably within a few hours. Initiating treatment after 72 hours of exposure is not recommended.
The decision to start PEP is made on the basis of degree of exposure to the HIV and the HIV status of the source from whom the exposure / infection has occurred. All the Medicines should be with casualty medical officer and he should be available round the Clock.

- Similarly if patient is HBV or HCV positive the appropriate authority should be informed and in a non immune health care worker(not previously immunized) hepatitis B vaccination should be started as soon as possible .If immunoglobulin for HBV is available it should be started within 7-8 hours.

Immune globulin and antiviral agents (e.g., interferon with or without ribavirin) are not recommended for PEP of hepatitis C. For HCV post exposure management, the HCV status of the source and the exposed person should be determined, and for HCW exposed to an HCV positive source, follow-up HCV testing should be performed to determine if Infection develops.

Steps recommended to be taken after Needle stick injury



6.2.3. Ingestion of potentially infectious material

- Protective clothing should be removed and medical attention sought.
- Identification of the material ingested and circumstances of the incident should be reported, and appropriate and complete medical records kept.

6.2.4 Potentially infectious aerosol release (outside a biological safety cabinet)

- All persons should immediately vacate the affected area and any exposed persons should be referred for medical advice.
- The laboratory supervisor or the bio-safety officer should be informed at once.
- No one should enter the room for an appropriate amount of time (e.g. 1 h), to allow aerosols to be carried away and heavier particles to settle.
- After the appropriate time, decontamination should proceed, supervised by the bio-safety officer.
- Appropriate protective clothing and respiratory protection should be worn.

6.2.5 Broken containers and spilled infectious substances

- Broken containers contaminated with infectious substances and spilled infectious substances should be covered with a cloth or paper towels.
- Disinfectant should then be poured over these and left for the appropriate amount of time (30 minutes).
- The cloth or paper towels and the broken material can then be cleared away; glass fragments should be handled with forceps.
- The contaminated area should then be swabbed with disinfectant.
- If dustpans are used to clear away the broken material, they should be autoclaved or placed in an effective disinfectant.
- Cloths, paper towels and swabs used for cleaning up should be placed in contaminated-waste container.
- Gloves should be worn for all these procedures.

6.2.6. Breakage of tubes containing potentially infectious material in centrifuges not having sealable buckets

- If a breakage occurs or is suspected while the machine is running, the motor should be switched off and the machine left closed (e.g. for 30 min) to allow settling.
- If a breakage is discovered after the machine has stopped, the lid should be replaced immediately and left closed (e.g. for 30 min).
- In either instance, the bio-safety officer should be informed.
- Strong suitable disposable gloves should be worn for all subsequent operations.
- Forceps, or cotton held in the forceps, should be used to retrieve glass debris.
- All broken tubes, glass fragments, buckets, and the rotor should be placed in a non-corrosive disinfectant known to be active against the organisms concerned. Unbroken, capped tubes may be placed in disinfectant in a separate container and recovered.
- The centrifuge bowl should be swabbed with the same disinfectant, at the appropriate dilution, and then swabbed again, washed with water and dried.
- All materials used in the clean-up should be treated as infectious waste.

. 6.2.7. Emergency services: whom to contact

The telephone numbers and addresses of the following should be prominently displayed in the facility:

1. The institution or laboratory itself (the address and location may not be known in detail by the caller or the services called)
2. In-charge of the institution or laboratory
3. Laboratory supervisor
4. Nominated Bio-safety officer (could be the laboratory supervisor himself/ herself)
5. Fire services
6. Hospitals/ambulance services/medical staff
7. Police
8. Medical officer
9. Water, gas and electricity services.

6.2.8. Emergency equipment

The following emergency equipment must be available:

1. First-aid kit, including universal and special antidotes
2. Appropriate fire extinguishers, fire blankets

Chapter 7

Transport of Infectious substances

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

Compliance with the rules will:

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

7.1 Precautions

Before transport, notify the receiving laboratory of all shipping and specimen details in advance of specimen arrival.

7.2 Surface transport/ courier service

- Securely fasten transport boxes in the transport vehicle.
- In the vehicle, keep a spill kit containing absorbent materials, chlorine disinfectant, heavy-duty reusable gloves, mask, and apron and leak proof waste disposal container.
- Arrange for an adequate amount of refrigerant (minimum of 4 ice packs will maintain refrigeration for 2-3 days) in case of delays in the travel schedule so that the cold chain is maintained.
- Avoid extensive vibration of samples, such as that encountered when traveling for long periods over rough roads as this can haemolyse samples, rendering them useless. If possible, separate the serum from clotted blood samples before transport.

7.3 Basic triple packaging system and maintenance of transit temperature

- The specimen should be transported in a basic triple packaging system (Fig 20) as described below to ensure bio-safety, transient temperature and quality of the specimen.

Triple vessel container has 3 components

7.3.1 Primary container

- The specimen is put in the labeled primary container which must be watertight, airtight, and wrapped with absorbent material (e.g. cotton wool)
- After tightening the cap, apply sealing tape to seal the cap.
- Put primary container in a separate plastic bag/ Ziploc bag.
- Two or more sealed specimens from the same patient may be placed in a larger plastic bag and sealed.
- Specimens from different patients should never be sealed in the same bag.

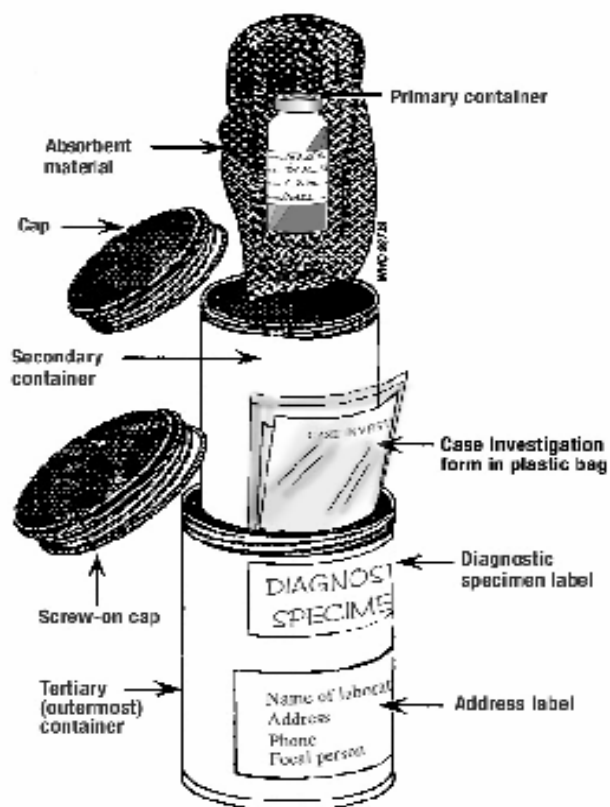


Fig 20: Triple vessel container

7.3.2 Secondary container

- Place the sealed bags containing the specimens inside secondary plastic containers with screw-capped lids. Provided the specimens have been double-bagged properly in sealed plastic bags, specimens from several patients may be packed inside the same secondary plastic container. Place additional absorbent material inside the secondary container to cushion multiple primary receptacles and absorb any leakage that may occur. Tape the laboratory request form sealed in a plastic bag to the outside of this secondary container.

7.3.3 Tertiary container

- The outer package or tertiary container protects the contents from physical damage and water while in transit. It should have a resistant, high-density external cover (e.g. metal, wood, or fireboard), shock-absorbent padding on the inside, and a tight-fitting lid. The outer package must be leak-proof and well insulated, and can contain ice, cold packs or dry ice when required. EPI vaccine carriers or other commercially made containers may be used as a tertiary container to transport. **Vaccine carriers that have been used for specimen transport must never be reused for carrying vaccines.**
- The rigid outer package is placed within an outer carton of double-ply corrugated cardboard or plastic, and a biohazard label is applied.

Biohazard label (Fig 21) should be put on packet/parcel containing infectious material for transport of samples

Fig 21



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

For further guidelines on collection, storage, labeling, packaging and transport of clinical samples including where to send the samples and what information to accompany the sample, please refer to the IDSP Laboratory manual for district laboratories, NICD 2004.

Chapter 8

Bioterrorism Agents- Laboratory Aspect

Biological weapons are device used intentionally to cause or death through dissemination of microorganisms or toxins in foods and water, by insect vectors or aerosols. Bioterrorism differs from other types of terrorism (chemical, radiological or nuclear).

Bioterrorism agents are highly infectious and all guidelines for biosafety should be strictly followed.

- Needs quick diagnosis as need is for specific & urgent action.
- Sample should be sent through courier on urgent basis to designated laboratory.
- Laboratory needs preparedness to handle large number of samples & transportations.
- Inform CMO on an urgent basis.

When to suspect that a disease out break is due to bioterrorism

- Drastic increase in microbiological culture request.
- Appearance of unusual sample
- Cluster of sample with same characteristics
- Diseases out break of same illness occurring in non contiguous area.

District laboratory needs to upgrade and develop rapid diagnostic techniques for such diseases which they can handle.

Types of waste item generated in a Diagnostic laboratory

SECTION	WASTE ITEM (S)
Sample collection	Sample collection vials, needles, syringes, cotton swabs, lancets
Bacteriology	Sample collection vials, test tubes, glass bottles, saline bottles, plastic bottles, testing containers, slides, infectious samples, bacteriological medium with growth, culture plates, blood, immersion oil and stains
Serology	Samples collection vials, blood, serum stored, tips, ELISA plates (TYPHIDOT plates), reagents
Clinical pathology	Urine and stool containers, reagents, slides, filter papers, wooden sticks, paper strips, chemical reagents

DISPOSAL OF LABORATORY WASTE ITEMS

WASTE ITEM	SUGGESTED METHOD FOR TREATMENT & DISPOSAL
Sharps like needles, lancets	<ul style="list-style-type: none"> Mutilate needle are using needle destroyer Collect in a puncture proof container Transfer to metal box Autoclave the metal box (Note: <i>In case needle destroyer is not available, destroy needle by burning the tip, put needle & syringe in 2% hypochlorite before autoclave</i>) Handover to a nearby central treatment facility. If not available, then dispose in burial pit/ landfill
Syringes	<ul style="list-style-type: none"> Mutilate and Immerse in 1% hypochlorite Microwave/shredder
Blood contaminated cotton swabs	<ul style="list-style-type: none"> Discard in a yellow bag Incinerator Discard as general non-infectious waste
<ul style="list-style-type: none"> Culture in plates/ bottle in liquid or solid media with bacterial growth specimens from patient 	<ul style="list-style-type: none"> collect in a stainless steel tray autoclave directly in disposal autoclave (holding time: 1 ½ hours at 15 lbs psi at 121 degree C empty the melted contents in a stainless steel bucket wash & reuse reusable plastic/ glass containers
<ul style="list-style-type: none"> glass slides 	<ul style="list-style-type: none"> autoclave discard as sharps
<ul style="list-style-type: none"> infectious liquid waste (blood/ plasma/ serum after sample analysis; liquid waste following routine washing procedure) 	<ul style="list-style-type: none"> decant carefully into a stainless steel container disinfect liquid waste following routine washing can be discharged in the drain
<ul style="list-style-type: none"> sample collection vials (blood/ serum/ plasma) 	<ul style="list-style-type: none"> sample vials with residual blood/ plasma are discarded in buckets containing 10 liter of 1 % hypochlorite immerse for 1 hour in hypochlorite or autoclave in disposal autoclave wash and reuse reusable containers drain residual hypochlorite into sink
<ul style="list-style-type: none"> sample collection containers (urine & stool) 	<ul style="list-style-type: none"> flush urine and stool in toilet discarded containers in hypochlorite autoclave the disposal container

Safety checklist

This checklist is intended to assist in assessments of microbiological laboratory safety and security status of biomedical laboratories.

Laboratory premises

1. Are the premises generally uncluttered and free from obstructions?
2. Are the premises clean?
3. Are there any structural defects in floors?
4. Is the working space adequate for safe operation?
5. Are the circulation spaces and corridors adequate for the movement of people and large equipment?
6. Are bench surfaces resistant to solvents and corrosive chemicals?
7. Is there a hand-washing sink in each laboratory room?
8. Are the premises constructed and maintained to prevent entry and harborage of rodents and arthropods?
9. Are all exposed steam and hot water pipes insulated or guarded to protect personnel?

Storage facilities

1. Are storage facilities, shelves, etc. arranged so that stores are secure against sliding, collapse or falls?
2. Are storage facilities kept free from accumulations of rubbish, unwanted materials and objects that present hazards from tripping, fire, explosion and harborage of pests?
3. Are freezers and storage areas lockable?

Sanitation and staff facilities

1. Are the premises maintained in a clean, orderly and sanitary condition?
2. Are hot and cold water, soap and towels provided?
3. Is there accommodation (e.g. lockers) for street clothing for individual members of the staff?
4. Is there a staff room for lunch, etc.?
5. Is there an adequate organization for the collection and disposal of general household rubbish?

Heating and ventilation

1. Is there a comfortable working temperature?
2. Are blinds or washable curtains fitted to windows that are exposed to full sunlight?

Lighting

1. Is the general illumination adequate?
2. Is task (local) lighting provided at work benches?
3. Are all areas well-lit, with no dark or ill-lit corners in rooms and corridors?
4. Are fluorescent lights parallel to the benches?

Services

1. Is there an adequate inspection and maintenance programme for fuses, lights, cables, pipes, etc.?

2. Are faults corrected within a reasonable time?
3. Are cleaning services available?
4. Is the access of cleaning personnel to various laboratory areas controlled and documented?
5. Are information technology services available and secured?

Laboratory bio-security

1. Is the whole building securely locked when unoccupied?
2. Are rooms containing hazardous materials and expensive equipment locked when unoccupied?
3. Is access to such rooms, equipment and materials appropriately controlled and documented?

Fire prevention and fire protection

1. Is there a fire alarm system?
2. Is the fire detection system in good working order and regularly tested?
3. Are all exits marked by proper, illuminated signs?
4. Are all exits unobstructed by decorations, furniture and equipment, and unlocked when the building is occupied?
5. Is access to exits arranged so that it is not necessary to pass through a high-hazard area to escape?
6. Do all exits lead to an open space?
7. Are corridors, aisles and circulation areas clear and unobstructed for movement of staff and fire-fighting equipment?
8. 13. Are laboratory rooms with potential fire hazards equipped with appropriate extinguishers and/or fire blankets for emergency use?
9. 15. Are personnel trained to respond to fire emergencies?

Electrical hazards

1. Does the interior wiring have an earthed/grounded conductor (i.e. a three-wire system)?
2. Are the flexible connecting cables of all equipment as short as practicable, in good condition, and not frayed, damaged or spliced?
3. Is each electric socket outlet used for only one appliance (no adapters to be used)?

Personal protection

1. Is protective clothing provided for all staff for normal work, e.g. gowns, coveralls, aprons, gloves?
2. Are safety glasses, goggles and shields (visors) provided?
3. Are there facilities for eye-wash?
4. Are there emergency showers (drench facilities)?

Health and safety of staff

1. Are first-aid boxes provided at strategic locations?
2. Are non-laboratory workers, e.g. domestic and clerical staff, instructed on the potential hazards of the laboratory and the material it handles?
3. Are notices prominently posted giving clear information about the location of first-aiders, telephone numbers of emergency services, etc.?

- a. Are women of childbearing age told that if they are, or suspect that they are, pregnant they should inform the appropriate member of the medical/scientific staff?
- b. Is there an immunization programme relevant to the work of the laboratory?
- c. Are skin tests and/or radiological facilities available for staff who work with tuberculous materials or other materials requiring such measures?
- d. Are proper records maintained of illnesses and accidents and reported to the designated officials?

Laboratory equipment

1. Are procedures available for decontaminating equipment prior to maintenance?
2. Are biological safety cabinets and fume cupboards regularly tested and serviced?
3. Are autoclaves and other pressure vessels regularly inspected?
4. Are centrifuge buckets and rotors regularly inspected?
5. Are pipettes used instead of hypodermic needles?
6. Is cracked and chipped glassware always discarded and not reused?
7. Are there safe receptacles for broken glass?
8. Are plastics used instead of glass where feasible?
9. Are sharps disposal containers available and being used?

Infectious materials

1. Are specimens received in a safe condition?
2. Are records kept of incoming materials?
3. Are gloves and other protective clothing worn for unpacking specimens?
4. Are personnel trained to ship infectious substances according to regulations?
5. Are work benches kept clean and tidy?
6. Are discarded infectious materials removed daily or more often and disposed of safely?
7. Are all members of the staff aware of procedures for dealing with breakage and spillage of cultures and infectious materials?
8. Is the performance of sterilizers checked by the appropriate chemical, physical and biological indicators?
9. Is there a procedure for decontaminating centrifuges regularly?
10. Are sealed buckets provided for centrifuges?
11. Are appropriate disinfectants being used? Are they used correctly?

Chemicals substances

1. Are incompatible chemicals effectively separated when stored or handled?
2. Are all chemicals correctly labelled with names and warnings?
3. Are chemical hazard warning charts prominently displayed?
4. Are staff trained to deal with spills?
5. Are flammable substances correctly and safely stored in minimal amounts in approved cabinets?

Needle stick sharp injury protocol

Name and Full address of Hospital _____

Needle Stick Sharp injury Protocol

Name of H.C.W. : _____

Section of HCW : _____

Employment No : _____

Date of Needle Stick /Sharp injury: _____

Date of Reporting of Casualty: _____

Site & Depth of injury: _____

Nature of injury: Needle Prick /Sharp Cut /Laceration /Splash of Fluids /Splattered Glass

Action taken in causality

Hep.B. vaccination given Yes / No

HBIG Yes / No

If Immunized: Date: _____ Intradermal /Intramuscular

Anti HBsAg Titer _____

HbsAg Positive / Negative

HIV antibody Positive / Negative

Information about Source of Contamination (If Available)

- Whether the patient has symptoms of HIV infection or no symptoms
- Serum sent for: (Reports to be entered in follow up visit)

01.Anti –HIV

02. HBs Ag

03. Anti –HCV

04. CD4 /CD8 counts

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