INTEGRATED DISEASE SURVEILLANCE PROJECT

TRAINING MANUAL FOR STATE & DISTRICT SURVEILLANCE OFFICERS

LABORATORY METHODS FOR CONFIRMATION OF DIAGNOSIS, COLLECTION, STORAGE, TRANSPORTATION OF SPECIMEN

Module – 6

CONTENTS

1. INTRODUCTION

This section covers

- Action to be taken by the HW, MO & lab assistant of the PHC, lab technician at district lab
- Collection, Preservation and Transportation of specimen
- Tests that should be conducted at various laboratories

2. SPECIFIC INSTRUCTIONAL OBJECTIVES

At the end of the session the participants would be able to

- ☞ List the L1 and L2 labs within the district; disease based L3 labs in the state and L4 and L5 labs in the country
- ☞ Understand the needs of the L1 and L2 labs (equipment, glassware, consumables, reagents and kits) and arrange for the logistic support to the labs.
- ☞ Identify what action is to be taken by the lab technician for sample collection in response to the diagnosis made by the MO
- ☞ List the tests that can be performed at L1 and L2 labs
- ☞ Identify the quality control process within the lab network
- ☞ Should be able to understand bio-safety issues
- ☞ To identify – transport modalities of sample to higher levels.
- ☞ Understand the training needs of the lab personnel.
- ☞ Keep a track of flow of samples
- ☞ To draw a flow diagram for reporting of the lab investigations

3. MODULE STRUCTURE AT A GLANCE

DURATION OF SESSION **2 HOURS**

S.No	CONTENT	METHODOLOGY	DURATION	TEACHING AIDS
1	Role of lab in disease surveillance. Core conditions under surveillance Organization of the labs	Lecture	30 mins	Power point
2	Disease specific action.Sample collection, storage and transportation; List of lab investigation at L1 and L2 lab	Module reading Discussions and questions	45 mins	Handouts and reading material
3.	Reporting formats	Lecture	20mins	OHP / PP
4.	Summation	Discussion, Group activity / Exercise	25 mins	Handouts

4. SALIENT POINTS TO REMEMBER

- Categorization of labs - List of L1 and L2 labs in the districts & List of Disease wise L3 labs in the state
- List of tests that can be done at L1 and L2 labs
- List of diseases that can be confirmed only by L3 labs
- Sourcing the consumables required by the labs
- Samples that have to be collected for specific disease
  - What sample
  - Quantity
  - Collection criteria
  - Transport and storage condition
- Bio Safety and waste management
- Quality assurance

5. FREQUENTLY ASKED QUESTIONS

- Who should initiate action for sample collection?
- What samples are to be collected for specific disease?
- To which level of lab should the samples be sent
- If the specific lab is not in a position to perform the test what action is to taken
- Who is responsible for providing the consumables etc to the lab? And from where they are to be obtained?
- In outbreak situation - action to be taken to involve the lab
- Training needs of the L1 and L2 lab technicians

6. GROUP ACTIVITIES

- Reading the handouts
- Discussions on the role and activity of lab
- Exercises

Exercise 1: Tick the action on each functionary outlined below

LEVEL	FUNCTIONARIES	DATA COLLECTION	SAMPLE COLLECTION	DATA ENTRY	TESTING	ANALYSIS	INVESTIGATION	RESPONSE	FEEDBACK
SUB DISTRICT LEVEL	LAB ASST								
	MO (PHC / CHC)								
DISTRICT LEVEL	DISTRICT LAB								
	DIST HOSP								
	MEDICAL COLLEGES								
	RRT								
STATE LEVEL	STATE SURVEILLANCE CELL								
	L3 LAB								
PRIVATE SECTOR	LABS								

Exercise 2: Fill in sample to be collected

Disease under surveillance	Sample to be collected	Test at L1 lab	Test at L2 lab
Malaria			
Tuberculosis			
Cholera			
Typhoid			
Leptospirosis			
Polio			
Dengue			
JE			
Measles			
Plague			

Exercise 3: Yes / No

Malaria	Microscopy at L2	
Tuberculosis	Microscopy at L1	
Measles	Serology at L1	
Dengue	Serology at L1	
Dengue	Serology at L3	
JE	Serology at L1	
Typhoid	Culture at L2	
Cholera	Culture at L2	

Exercise 4: Lab requirements: fill in

Equipments	
Glass ware	
Reagents for culture	
Biological reagents: Antiserum and standard cultures	
Stains for malaria and TB microscopy	
Rapid response action	

Exercise 5: List the purpose for which the form is used

Lab request form	
Lab form L1	
Referral form	

Exercise 6: Transportation of specimens (where and how)

Stool for Cholera culture	
Blood for Typhoid culture	
Serum for Leptospirosis	
Nasopharyngeal swab for measles virus isolation	

TASKS AT EACH LEVEL; *IMPLIES AS PER THE GUIDELINES OF THE VERTICAL PROGRAMMEMES

Disease under surveillance	Tasks at Level 1	Level 2	Level 3
Malaria*	1.Blood Sample collection 2.Smear preparation 3.Microscopy and reporting	1.Same as L1 2.QC of L1	QC of L2
Tuberculosis	1. Sputum collection 2.smear preparation 3.Microscopy and reporting 4.Transport to L3 for culture	1. Same as L1 2.QC of L1 3. Transport to L3 for culture	1.Culture and Sensitivity testing 2.Quality control
Cholera	1.Stool sample collection 2.Transport to L2	1.Stool sample 2.Microscopy 3.Culture 4.Biochemical & serotyping 5.Transport to L3 for sensitivity	1.Training 2. Drug sensitivity and Phage typing 3.QA of L2
Salmonellosis	1.Blood and stool collection for culture 2.Typhidot test 3.Transport to L2	1.Widal test 2. Typhidot 3. Blood and stool culture 4. QA of L1	1.Training 2.QA 3.Special tests
Leptospirosis	1.Collection of blood and urine 2.Transport to L2	1.Dark Field Microscopy 2.Serology by latex agglutination/ IgM ELISA 3.Transport to L3 for culture	1.Culture 2.MAT test & Serovar identification
Polio*	Sample collection and transport to designated labs as per the NPSP guidelines	Sample collection and transport to designated labs as per the NPSP guidelines	Sample collection and transport to designated laboratories as per NPSP guidelines
Dengue	1.Collection of blood for serology and for virus isolation 2.Transport to L2	1.Serology by Elisa/ or rapid method 2.Transport to L3 for culture	1. Culture to be performed in a designated lab (which needs to be defined as a disease-specific L3 or L4/L5 labs 2.Serology by IgM ELISA and rapid tests 3.QC for L2 lab
Japanese encephalitis	1.Collection of samples for serology and culture 2.Serum separationTransportation of samples to L3	1.Same as L1	1.Serology to be performed in a designated lab (which needs to be defined as a disease-specific L3 or L4/L5 labs due to their problem of availability of kits
Measles	1. Collection of blood and urine samples 2.transport to L3	Same as L1	1. Virus culture in designated lab.
Anthrax	1. Information to L2	1.Specimen Collection and transportation to L3	1.Culture
Rickettsial diseases	1. Collection of blood and transportation of serum to L2	1. Weil-Felix test 2.transport to L3	1Weil-Felix test 2. Confirmation of diagnosis to be performed in a designated lab (which needs to be defined as a disease-specific L3 or L4/L5 labs
Plague	1.Assist in sample collection	Staining and microscopy Transport sample to L3 lab no reporting	Culture, serology and confirmation to be performed in a designated L4/ L5 labs
Hepatitis	Collect and send sera to L2	Rapid test for Hepatitis B, C if availableTransport sample for A,D, E and other markers to L3	1.QC of L2 2. Serology for all Hepatitis markers
Water Quality	1.Collection of samples 2.Rapid test-H_2S strip	1.Collection of samples 2.Rapid test- H_2S strip 3. MPN test	1. Same as L2 2. QC for L2

*Since plague is a notifiable disease, the sample should be collected and sent to referral laboratories:
1. Central Plague Laboratory, Zoonosis Division, National Institute of Communicable Diseases, 22, Sham Nath Marg, Delhi-110054, Tel: 011-23912901, 011-239123148
2. Plague Surveilance Unit, NICD, NTI Campus, 8-Bellary Road, Bangalore, Tel: 080-23446723.

7. HANDOUT ON ROLE OF LABORATORIES IN IDSP

7.1 Introduction

Laboratory support to disease surveillance activities has been recognized as an essential component of any surveillance programmeme both for communicable as well as non-communicable diseases.

Laboratory based disease surveillance will be the third level of surveillance in IDSP. The laboratories will assist in passive routine surveillance of selected diseases and active surveillance in case of outbreak investigations. The laboratories will participate in:

- ☞ Early confirmation of diseases under surveillance
- ☞ Epidemiological investigation
- ☞ Rapid laboratory confirmation of the diagnosis
- ☞ The implementation of effective control measures

The laboratory network for IDS will be established at four levels of functions. It will include both private and government labs:

- ♦ Peripheral Laboratories and Microscopic centers - L1 Labs
- ♦ District Public Health Laboratory - L2 Labs
- ♦ Disease Based State Laboratories - L3 Labs
- ♦ Regional Laboratories IDSP and Quality control Laboratories - L4 Labs
- ♦ Disease based reference Laboratories - L5 Lab

* a glossary should specify the participating laboratories at each level. At the peripheral level e.g. PHC lab, Microscopy centers, CHC labs and private labs (if any). Similarly at each of the other levels the final list of category of participating laboratories can be included only when an understanding has been reached with each of them.

The laboratory results are used to accurately diagnose the patient so that appropriate therapy can be given, as well as verify the cause of suspected outbreaks. As a Public Health Manager, one needs to identify what samples are to be collected and where they have to be sent, so that there is minimum delay in confirming the diagnosis.

The initial investigation involves two important processes: collection of information on suspect cases and collection of clinical specimens for laboratory diagnosis.

Successful laboratory confirmation of a disease depends on:

- ☞ Advance planning
- ☞ Collection of appropriate and adequate specimens
- ☞ Correct packaging and rapid transport to an appropriate laboratory
- ☞ The ability of the laboratory to accurately perform the diagnostic tests
- ☞ Proper bio safety and decontamination procedures to reduce the risk of further spread of the disease

Consider the logistic requirements for sampling equipment and supplies, specimen handling and transport to the laboratory (timing, route, transmit temperature requirements, shipping procedures, and documentation), and decontamination procedures in advance.

7.2 METHOD OF LABORATORY SURVEILLANCE

Laboratories will be participating in:

Routine passive surveillance of the selected diseases: In case of fever and cough of more than 3 weeks the field worker would initiate the sample collection as per the respective programme requirements (Tuberculosis). In other syndromic presentations the health worker would refer the case to the MO of the PHC for further investigation.

In case of an outbreak situation, the MO with the help of the surveillance team would initiate action including sample collection. Once a suspected outbreak has been detected and reported, an epidemiological investigation would be quickly organized. Several key issues will be discussed, and decisions agreed upon before the team goes to the field. Ultimately these decisions will guide the materials and procedures required for efficient specimen collection and their transport to the laboratory for testing.

Core Conditions under surveillance in IDSP

(i) Regular Surveillance:

Vector Borne Disease	: 1.	Malaria
Water Borne Disease	: 2.	Acute Diarrhoeal Disease (Cholera)
	: 3.	Typhoid
Respiratory Diseases	: 4.	Tuberculosis
Vaccine Preventable Diseases	: 5.	Measles
Diseases under eradication	: 6.	Polio
Other Conditions	: 7.	Road Traffic Accidents (Linkup with police computers)
Other International commitments:	: 8.	Plague
Unusual clinical syndromes (Causing death / hospitalization)	: 9.	Meningoencephalitis/Respiratory Distress, Hemorragic fevers, other undiagnosed conditions

(ii) ***Sentinel Surveillance***

Sexually transmitted diseases/Blood borne	: 10.	HIV/HBV, HCV
Other Conditions	: 11.	Water Quality Monitoring
	: 12.	Outdoor Air Quality (Large Urban centers)

(iii) ***Regular periodic surveys:***

NCD Risk Factors	: 13.	Anthropometry, Physical activity, Blood Pressure, Tobacco, Nutrition, Blindness

(iv) Additional State Priorities : Each state may identify up to five additional conditions for surveillance.

- ☞ The number of core diseases is limited to improve quality of surveillance and to reduce workload on the peripheral health worker.
- ☞ The list will be reviewed and modified according the needs of surveillance at least once in 2 years.

7.3 ORGANIZATION OF LABORATORIES

Stratification of laboratories

The laboratory network for IDSP will be established at five levels of functions based on location of lab, expected functions and facilities available. A national agency will be designated as the coordinated agency, which shall also undertake monitoring and quality assessment of the laboratories at peripheral and intermediate levels. All laboratories will supervise their immediate lower level labs. * peripheral laboratories may include PHC, block PHC, CHC and select private laboratories (if any). Similar listing in the glossary for other level labs. The list at other levels can only be finalized after discussionswith the core group because it would require the consent of the individual labs i.e. L3, L4and L5 labs

- Peripheral Laboratories - L1 labs
- District hospital/Public Health Laboratory - L2 labs
- Disease Based State Laboratories - L3 labs
- Regional Quality Control Laboratories - L4 labs
- Disease based National reference Laboratories - L5 labs

TASKS AT EACH LEVEL

Disease under surveillance	Tasks at Level 1	Level 2	Level 3
Malaria	1.Sample collection 2.Smear preparation 3.Microscopy and reporting	1.Same as L1 2.QC of L1	QC of L2
Tuberculosis	1. Sputum collection 2.smear preparation 3.Microscopy and reporting 4.Transport to L3 for culture	1. Same as L1 2.QC of L1 3. Transport to L3 for culture	1.Culture and Sensitivity testing 2.Quality control
Cholera	1.Stool sample collection 2.Transport to L2	1.Stool sample Microscopy 2.Culture 3.Biochemical & serotyping 4.Transport to L3 for sensitivity	1.Training 2. Drug sensitivity and Phage typing 3.QA of L2
Salmonellosis	1.Blood and stool collection for culture 2.Typhidot test 3.Transport to L2	1.Widal test 2. Typhidot 3. Blood and stool culture 4. QA of L1	1.Training 2.QA 3.Special tests
Leptospirosis	1.Collection of blood and urine 2.Transport to L2	1.Dark Field Microscopy 2.Serology by latex agglutination/ IgM ELISA 3.Transport to L3 for culture	1.Culture to be performed in a designated lab (which needs to be defined as a disease-specific L3 or L4/L5 labs 2.MAT test & Serovar identification to be performed in a designated lab (which needs to be defined as a disease-specific L3 or L4/L5 labs
Polio	Sample collection and transport to designated labs as per the NPSP guidelines	Sample collection and transport to designated labs as per the NPSP guidelines	designated labs as per the NPSP guidelines
Dengue	1.Collection of blood for serology and for virus isolation 2.Transport to L2	1.Serology by Elisa/ or rapid method 2.Transport to L3 for culture	1. Culture to be performed in a designated lab (which needs to be defined as a disease-specific L3 or L4/L5 labs 2.Serology by IgM ELISA and rapid tests 3.QC for L2 lab
Japanese encephalitis	1.Collection of samples for serology and culture 2.Serum separationTransportation of samples to L3	1.Same as L1	1.Serology to be performed in a designated lab (which needs to be defined as a disease-specific L3 or L4/L5 labs due to their problem of availability of kits
Measles	1. Collection of blood and urine samples 2.transport to L3	Same as L1	1. Serology by IgM ELISA virus culture in designated lab.
Anthrax	1. Information to L2	1.Specimen Collection and transportation to L3	1.Culture
Rickettsial diseases	1. Collection of blood and transportation of serum to L2	1. Weil-Felix test 2.transport to L3	1Weil-Felix test 2. Confirmation of diagnosis to be performed in a designated lab (which needs to be defined as a disease-specific L3 or L4/L5 labs
Plague	1.Assist in sample collection	Staining and microscopy Transport sample to L3 lab no reporting	Culture, serology and confirmation to be performed in a designated L4/ L5 labs
Hepatitis	Collect and send sera to L2	Rapid test for Hepatitis B, C if available Transport sample for A,D, E and other markers to L3	1.QC of L2 2. Serology for all Hepatitis markers
Water Quality	1.Collection of samples 2.Rapid test-H_2S strip	1.Collection of samples 2.Rapid test- H_2S strip 3. MPN test	1. Same as L2 2. QC for L2

REQUIREMENTS OF L1 LABS

Infrastructure	Space for microscopy, work table for test procedures, washing, sterilization and decontamination
Equipments	
Microscope, binocular with ain built liquid attachment	1 no
Autoclave for sterilization	1 nos
Autoclave for decontamination	1 No
Micropipettes 50-200 μl, 100-1000 μl	One each
Centrifuge (benchtop)	1 no
Refrigerator	1 no
Water bath	1 no
Consumable Items	Approx. quantity required per year
Autoclavable polypropylene items	
Screw capped round bottom 5 ml vials externally threaded	500 nos
Screw capped round bottom 12 ml vials	500
2ml screw capped Storage containers externally threaded	1000
Test tube racks	4 nos
TT racks for 5 ml tubes	2 nos
Micropipette tips 50-200 microliters (yellow color)	1000 nos
Slide boxes for 25 slides	5 nos
Wash bottles	2 nos
Stool collection bottles (presterilized)	200 nos
Urine collection bottles (presterilized)	50 nos
Sputum collection containers	100 nos
Gloves autoclavable	200 nos
Vaccine carrier with ice packs	4
Glass items	
Measuring cylinders – 100 ml, 50 ml	2 nos each
Conical flask –100 ml	3 nos
Conical flask –200 ml	4 nos
Pasteur pippettes (disposable, presterilized, individually wrapped)	1000nos
2ml, 5ml and 10ml pipettes (glass)	Each 10 nos
Others	
Fine tips forceps	2 nos
Lancet	50 nos
Autoclave labels	Quantity would depend on number of tests and would vary. The lab in charge / MO could give the annual requirements to the DSO who would procure it through the identified modalities.

Reagents	
Stain for Malaria and TB	
Typhidot test kit	
H2S testing kit	
Stationary, records and report forms	
Requirements of L2 labs	
Infra Structure	Space for washing, sterilization, decontamination, Media reparation, worktables, Safety cabinets, culture facility, ELISA, other serology and office
Equipments	Number required
Autoclave for sterilization	1
Autoclave for decontamination	1
Hot air oven	1
Incubator	1
Water bath	1
Weighing balance	1
Microscopes (Binocular)	2
Vertical Laminar flow cabinet	1
ELISA reader and washer	1
Micro plate shaker	1
Refrigerators	2
-20 deep freezers	1
Centrifuge	1
Cyclomixer	1
Hot plate	1
Distilled water still	1
pH meter	1
Variable volume Micro pipettes 50-200 µl, 100-1000 µl	One each
LPG cylinders, regulators and burners	2
Needle shredder	1
Mini Incinerator	1
Consumables and Glass Ware	**Approximate / annum**
Disposable syringes with needles	1000
Polypropylene storage vials	500 each
10 ml and 15 ml ml test tubes (glass/ PP)	500 each
Sterile sputum containers	250
Sterile Urine containers	100
Sterile stool sample containers	200

2, 5, 10 ml pipettes (glass)	50 each
Pasteur pipettes presterlized individualy wrapped	250
Measuring cylinders – 50,100, 250, 500, and 1000 ml	Each 2
Flasks – 60, 100, 250, 500 & 1000 ml	Each 5
Petri dishes	100
Reagent bottles – 60 and 100 ml	Each 4
Beakers – 50, 100, 200 ml	Each 6
Blood culture bottles (100 ml)	50
Centrifuge tubes – 15 ml	25
Funnel – dia 4 and 6 inches	Each 3
Lancet	25
Inoculating loops	12
Test tube racks (18 hole)	10
Micropipette tips 100 microlitre	500
Variable Micropipette tips 100 – 1000 microlitre	500
Slides & Slide Boxes	4
Gloves – varying sizes	Each 50
Tourniquet	3
Surgical masks	500
Discarding bags	500
Vaccine carrier with ice packs	4
Absorbent material - cotton	Quantity has to be determined based on anticipated number of cases / annum. (Variable from dist to dist). The lab in charge / MO could give the annual requirements to the DSO who could procure it through the identified modalities.
Labels	
Glass marking pens	
Adhesive tape	
Scissors	
Scalpel and blades	
Forceps	
Rubber teats	
Thermometer	

MEDIA AND REAGENTS

Dehydrated Culture Media Mac Conkey, and broth	**Stains** - JSB, Ziehl Neelson'snelson, Grams, Waysons,
TCBS agar	**Kits** – Typhidot Dengue water testing, Hepatitis, Weil Felix,
Biochemical test media	**Chemicals** for reagent and media preparation
Cary Blair media	
Nutrient Agar	
Mueller Hinton agar	**Miscellaneous**
Selenite F enrichment broth	Hand disinfectant – Savlon, dettol
Antibiotic discs	Disinfectant solution – Sodium
Specimen collection bottles	Hypochlorite 4%
XLD MediaPeptone	Autoclave and Hot air oven indicator tapes
Kovac' s reagent	Autoclave spore strips
Oxidase disc	**Stationery**:
HCL H_2SO_4	Lab request forms, Records etc
NaOH	
Widal reagents	Packing material like aluminium for
Anti sera – Cholera	Special Brown Paper, Twine ball
Standard reference material such as standard bacterial strains	

The District surveillance officer is responsible for the L1 and L2 labs within the district.

Functions of L1 lab technician:(The MO to assist in blood collection)

1. Collection of samples for investigations
2. Perform the quality Laboratory tests assigned to the L1 level.
   a. Microscopy for Malaria
   b. Microscopy for TB
   c. Typhidot test for Typhoid fever
   d. H_2S test for water quality.
3. Transport of relevant sample to L2 lab for culture and other serological investigations
4. Assist rapid response team in sample collection

5. Participate in external quality assurance conducted by L2 lab

Functions of L2 lab technician

1. Perform all the tests performed by L1 lab
2. Quality assurance of L1 lab
3. Perform lab test assigned to L2 level
    a. Culture & sensitivity tests for Cholera,
    b. Serological tests for Cholera, Typhoid, Dengue, Leptospirosis
    c. MPN test for water quality
4. Transport relevant samples for testing at L3 lab
5. Transport 5% of tested samples to L3 for testing and QA
6. Reporting test result to L1 lab for sample received from L1 lab
7. Reporting test result weekly to DSO

7.4 Biosafety in Laboratories

Good laboratory technique is fundamental to laboratory safety. Important concepts to have lab safety are listed below.

- Entry / access to laboratory area
- Have a biohazard sign (Fig) displayed on the doors of the rooms where infectious agents are handled.

Fig: Biohazard sign.

- Entry to laboratory working area should be only for laboratory persons.
- Doors to the laboratory should be kept closed.
- No smoking, eating, or drinking is allowed in laboratory area.

Personal Protection

- While working in the laboratory always wear lab coat.
- Have all the personnel protective equipments ready & use them as per the procedures strictly for highly infectious diseases outbreaks.

- Wear gloves for all procedures that may involve direct or accidental contact with blood / infectious materials.
- After use, gloves should be removed carefully without touching infected surface, disposed off in container containing disinfectant solution. Hands should be washed with soap & water.
- Laboratory personnel must wash their hands after handling infectious materials/ performing test procedures and before they leave the laboratory working area.
- Laboratory coat should not be worn outside the laboratory area i.e canteen, library, and toilet or staff common room.
- Eating, drinking, applying cosmetics and handling contact lens are strictly prohibited in the laboratories.
- Laboratory coat used/unused should not be placed in the same cupboard with street clothes or food articles etc.
- Lab personnel should receive suitable vaccination e.g. Hepatitis B.

General procedural precautions

- Mouth pipetting must be strictly avoided.
- Materials / articles must not be held in the mouth. Do not lick / wet labels for sticking.
- All technical procedures should be such that they minimize the formation of aerosols and droplets. In the district laboratories do not perform any procedure that generates lots of aerosolization unless there is an access to biological safety cabinet.
- Do not use hypodermic needles and syringes for pipetting devices.
- All spills, accident or exposure to infectious materials must be reported to laboratory in charge and a record should be maintained.
- Display written procedures for the clean up of all spills.

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.

Laboratory working areas

- Keep the laboratory area neat, clean and free of materials that are not required.
- Decontaminate the working surface after any spill and at the end of the working day using 1 % Sod. hypochlorite.
- All contaminated materials, specimens, cultures, must be decontaminated in the laboratory premises before final disposal or cleaning for reuse.
- If there are windows in laboratory area, they should have arthropod / mosquito & fly proof mesh.

Bio safety Management

- Have one person responsible for bio safety activitiesà Biosafety officer.
- Health checks up of laboratory staff at regular intervals.
- Immunization against diseases which are feasible must be given regularly, especially Hepatitis B.
- Bio safety officer should train lower staff regularly.

Laboratory Designs and Facilities

- **Design**
- Enough space should be available
- Smooth easily cleanable walls, ceiling and floors which should be impermeable to liquids and resistant to chemicals and disinfectants.
- Ample illumination should be available for safe conduction of laboratory procedures.
- Regular, continuous and dependable, quality water supply should be available which is important for laboratory techniques.
- Wash basins with running water if possible should be provided in each laboratory room preferably near the exit door.
- Suitably equipped first aid box should be available in the district laboratory.
- Control programmeme for rodents and insects in the laboratory should be there.

Laboratory Equipment

- ☐ Ensure the adequate equipment be provided and that they are used properly.
- ☐ **Essential biosafety equipment are**
    - Pipetting aids to avoid mouth pipetting
    - Screw capped tubes and bottles.
    - Autoclaves to decontaminate infectious material wastes.

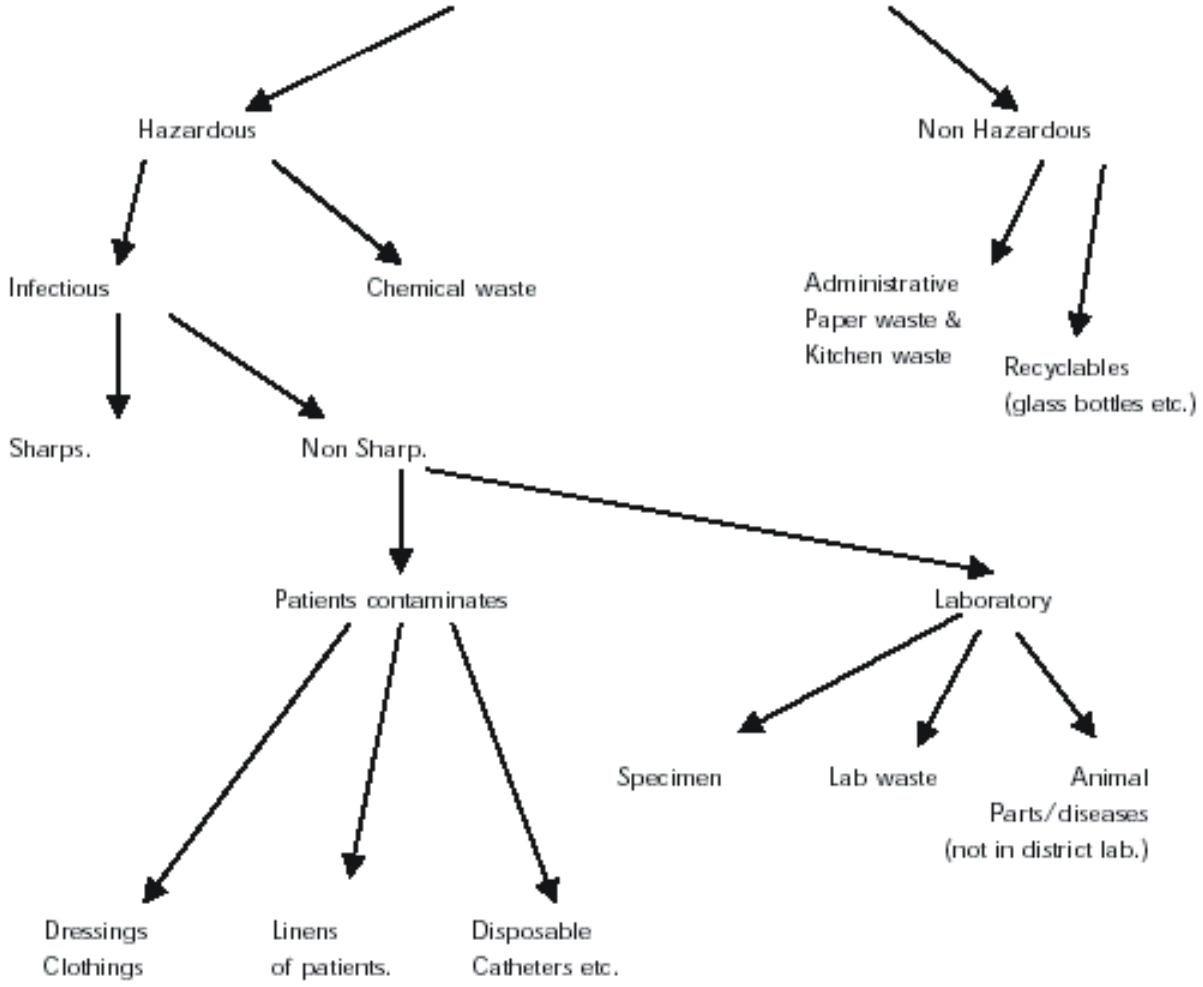
- Plastic disposable pasteur pipettes, when ever possible should be used.
- Equipments should be validated before being taken for use and then revalidation should be done at regular intervals.

7.5 Waste Management

What is Waste?

Any thing which has to be discarded is called waste. The laboratory organisms require appropriate handling. The most common documented transmission of infection from waste to health care worker is through contaminated metallic waste.

Fig;3.4 - Classification of Laboratory Waste / Hospital wastes.



Hospital/Laboratory Waste Management

- **Material required**
- Waste disposal color coded bags with biohazard symbol. blue, red, black and yellow.
- Trolley baskets for holding the bags.
- Autoclave for decontamination of waste on site.
- Disinfectant solution (Sodium hypochlorite solution.).
- Incinerator if possible (Optional).
- Soap for hand washing and towel for drying hands.
- Gloves.
- Puncture proof containers plastic / metal with a biohazard symbol.

Follow management at every step from the site of generation

- Segregation.
- Collection.
- Transportation.
- Storage.
- Treatment to disinfect.
- Final disposal.
- Segregate waste into the prescribed categories at the point of generation.
- Color coded bags as per international norms. (Table-3.1)

Table-3.1 Container and color coding for disposal of bio-medical lab wastes

Waste category	Waste class.	Type of containers	Colorcoding	Treatement of waste Disposal.
1.	Microbiology & Biochem. Lab.	Plastic holding bags with biohazard sign.	Yellow	Autoclaving/ Microwave & shredding.
2.	Waste sharps	Reusable plastic/ Metal containers	Blue	Shredding & deep buried.
3.	Discarded chemical reagents, kits.	-------Do-----	--Do--	---------Do----------
4.	Soiled wastes (Lab coats etc.)	Plastic bags with biohazard sign.	Yellow/Black.	Disinfect / Autoclave then Machine wash.
5.	Chemical wastes	Sturdy containers or Plastic holding bags.	Yellow/Black.	Incineration (not Mercury)
6.	Disposable other than sharps.	Reusable sturdy containers/Plastic bags.	Yellow/Black	Disinfect/ Autoclaving/ shredding /buried.

Methodology

In the district lab, the lab waste handling is an essential job which needs to be under supervision of biosafety officer. Broad guidelines to be followed are:

- Segregate the different category of waste at the point of generation.
- Discard infectious wastes (non sharp) if possible in disinfectant solution or autoclave to render it non-infectious.
- Discard sharp waste i.e. needles, blades etc in a puncture proof containers.
- After the container is 2/3 filled, it should be autoclaved/ shredded and land filled for disposal.
- If nothing is available for disposal deep bury (as per standard guidelines) in a secure area.

Categories

All waste should be decontaminated (chemically/autoclaving) before final disposal/ reuse.

- Non contaminated waste which can be reused or recycled, disposed off as general house hold waste.
- Contaminated sharps disposed off in puncture proof containers fitted with cover, labeled as infectious.
- Contaminated reusable materials for decontamination by autoclave, thereafter washing and reuse/ recycle.
- Contaminated disposable material for autoclaving & disposal.
- Contaminated material for direct incineration.

Quality control

- Check that proper quality bags are purchased.
- Autoclave monitoring & maintenance.
- Disinfectant quality check.

Contaminated infectious materials for autoclaving and reuse

- No pre cleaning to be done.
- Transfer material to autoclave.
- Autoclave at 121°C / 15 lbs pressure for 45 minutes.

- If cleaning is required, do washing as prescribed.
- Re use.

Contaminated infectious waste for disposal

- Autoclave in leak proof container. i.e. autoclavable colour coded plastic bags.
- Place material in a transfer containers / trolleys with bags.
- Transport to incinerator.
- If reusable transfer containers are used they should be disinfected and cleaned before they are returned to laboratories.
- Discarding jars preferably unbreakable should be used and they should have suitable disinfectant (Sodium hypochlorite 1%) freshly prepared each day.

Sodium Hypochlorite Solution Preparation

Dilution of sodium hypochlorite solutions (part of stock solution: parts of water)

Required Strength	4% Stock Solution	5 % Stock Solution	10% Stock Solution	15% Stock Solution
0.1% (1g/L-1000 ppm)	1:40	1:50	1:100	1:150
0.5 % (5g/L-5000 ppm)	1:20	1:25	1:50	1:75
1% (10g/L-10.000 ppm)	1:4	1:5	1:10	1:15

Note:- Alaways prepare diluted hypochlorite solution fresh every day. If sodium hypochlorite is not available an alternative calcium hypochlorite (1%) can be used which needs to be prepared as follows

CALCIUM HYPOCHLORITE SOLUTION

Chlorine available in powder form	How to dilute to 0.1%	Chlorine available in 0.1% solution	How to dilute to 1%	Chlorine available in 1% solution
35%	2.8 gms to 1 litre in water	1000 ppm	28 gms to 1 litre in water	10000 ppm

7.6 Quality Assurance

General principles and procedures:

Quality assurance is the sum total of all activities that are undertaken to ensure generation of reliable and accurate results / data. It is concerned with the organizational process and the conditions under which laboratory activities are planned, performed,

monitored, recorded and reported. The basis for any clinical laboratory quality control programme is a written QC policy. This policy should include a description of:

a. The control materials to be used with each test

b. Frequency of use,

c. Required documentation as well as criteria for the acceptability of patient's results. The quality control policy must also state that when results are unacceptable, any patient's results that were done with those QC specimens cannot be reported. It should identify a set of remedial actions that should be instituted.

Following are the objectivities of Laboratory Quality Assurance

- ☞ Maintain efficiency through standard procedures.
- ☞ Maintain accuracy, consistency and reliability of results and data.
- ☞ Prevent risks
- ☞ Detect deviations and Correct errors
- ☞ Accreditation of the laboratory

It is the responsibility of the head of the laboratory to establish, implement and ensure compliance. However, Laboratory Quality Assurance (LQA) is the responsibility of all laboratory personnel. The DSO and the MO should ensure that the lab maintains all records pertaining to the quality checks.

Quality assurance comprises of internal quality control and external quality assessment.

Internal Quality Control

For qualitative tests, the quality control rules are simple.

1. The positive control must be positive and the negative control must be negative. For the patient's results to be valid a quality assurance programme is in place to ensure the reliability of test results
2. Testing should be processed in an orderly, reproducible fashion in a setting where environmental variation will not affect the result
3. All extraneous variables must be excluded
4. Records are available to document

To achieve and maintain IQC the following should be checked and followed:

1. Test Request and Specimen Collection

Tests should be ordered on a request form that includes the patient's name and a unique identifier, the time of collection, and the nature of the specimen. It is good practice to keep a log of all specimens collected, including the time of collection and, in the case of bacteriologic specimens, the source of the specimen. All specimen containers and tubes must also be marked with the patient's name and unique identifier, preferably at the time and place of collection.

2. Test Processing

- **Temperature monitoring:** The temperature in the testing environment, as well as in refrigerators, freezers, incubators, water baths involved in testing or storing reagents or media, should be monitored daily and the results

recorded. If storage conditions for reagents or media are disturbed, as in a power outage with a rise in storage temperature, the reagents must be considered unreliable and discarded, even though the expiration date has not yet been reached.

- **Reagents used:** All working solutions, prepared from more concentrated stock solutions, should be labeled when they are prepared and the expiration date written clearly on the label if appropriate.
- **Maintenance of equipments**: For reliable results, all microscopes, centrifuges and other laboratory equipment must be properly maintained. In taking responsibility for the validity of the test information produced in the office laboratory an equipment log should be kept that keeps track of maintenance, problems found, and any corrective action taken. Refrigerators, freezers, incubators, and heating baths are a class of equipment that can directly affect test reliability and should thus be included in the general maintenance programme.

Small pieces of equipment, such as pipettes, should be stored or marked in such a way that there is little chance of inadvertently using the wrong size (e.g., a 1-mL pipette instead of a 0.5-mL pipette), and damaged pieces should be discarded. The pipetting and diluting step is one of the most critical in many procedures and requires extreme care on the operator's part.

3. Reporting and Using Test Results

The other important phase of laboratory testing is accurately reporting the results in a timely manner with appropriate interpretation and application of the test results.

All laboratory results should include information similar to that required for ordering the test: patient identification, time and date of specimen collection, body site of collection, and name of test. In addition, the test result report needs to contain: test results including the units of measurement, reference intervals (normal values), time and date of analysis, Reports should be written on a form designed for that purpose.

Good laboratory practice requires that laboratories routinely check for clerical errors prior to the release of laboratory results. Usually this calls for a second person to read the test result on the report to see if it is reasonable

Timeliness in reporting is just as much a quality issue as are the accuracy and precision of testing.

External Quality assurance at 2 levels

☞ Within the state IDSP system

☞ Through an external agency not associated with the system

Within the state IDSP system

☞ Quality control of the peripheral laboratories will be the responsibility of the district public health laboratory. Confirming 5-10% of laboratory results of PHC laboratory should be done at the district laboratory.

- ☞ Quality control of district labs (L2) is the responsibility of L3 labs. Confirming 5-10% of laboratory results of L2 laboratory should be done at the state laboratory

Laboratory manuals for work on specific diseases will be made available to peripheral laboratories, both private and government, under IDSP programme. Training, on job evaluation and retraining will be provided to the laboratory personnel as part of quality assurance.

External quality assurance by an agency not associated with the system

The Institution conducting EQAS will be identified by the government and intimated to the lab. The lab will follow instructions of the National EQAS center.

Activities under EQAS Program

The laboratory diagnoses on the listed communicable diseases and non-communicable diseases (NCDs to be lab tested only at the state lab level) will be evaluated in this programme.

Frequency of Tests and Number of tests will be decided by the agency

Evaluation of the labs and action to be taken will be decided The final report will identify laboratories as consistent poor performers, poor performers correcting errors, inconsistent performers, good performers with subsequent poor performance and consistent good performers. A critical evaluation enumerating the causes and reasons for such categorization will be part of the final report. This helps the state take appropriate corrective action.

Specimen collection and transport

Peripheral blood smears, sputum for smear and culture, blood for serology, blood, stool, pharyngeal / nasopharyngeal swabs and urine for culture and water for water quality test are the common specimens to be collected under IDSP. In outbreak conditions, the Rapid Response Team will order for appropriate samples. (At L1 level the lab assistant is trained in collection of blood by fingerpicks for smear preparation, collection of blood by venepuncture would require the assistance of the medical officer).

General instructions

1. Use universal precautions for handling all specimens.
2. Whenever possible, collect all culture specimens prior to administration of any antimicrobial agents.
3. All specimens must be appropriately labeled with the requisition number from the corresponding requisition. The request form will include the patient name, hospital /op number, date and time of collection, specimen type and tests requested. A requisition needs to accompany each different specimen type.
4. Specimens should be in tightly sealed, leak proof containers. Specimens should not be externally contaminated. Specimens grossly contaminated or compromised may be rejected.
5. If unable to transport specimens promptly, refrigerate serum, urine, respiratory samples and stool specimens. Leave blood culture bottles at room temperature.

7.7 Sample Collection*

Summary of Sample Collection and Transportation

SPECIMEN	COLLECTION	TRANSPORTATION
Blood for smears	Capillary blood from finger prick. Make smear, fix the same in methanol or other fixative.	Transport the slides within 24 hours. They must not be refrigerated.
Blood for culture	Venous blood 0.5 – 2 ml for infants 2 – 5 ml for children 5 – 10 ml for adults	Collect into blood culture bottles (With Glucose broth or Bile salt broth) Transport in erect position, and with enough cushion to prevent lysis of cells. Wrap tubes with absorbent cotton to soak any spillage. If specimens can reach a lab within 24 hours, then it can be sent in ambient temperature. Else cold chain at 4*C.
Serum	Venous blood is collected and placed in a sterile test tube. Let the specimen clot for 30 minutes at ambient temperature. Then place in a cool box for clot retraction at 4 – 8*C, for a minimum of 1 – 2 hours. This is then centrifuged @ 1000 G for 10 mts[1]. Separate the serum from the clot.	Sera should be transported at 4 – 8*C and Can last at this temperature for up to 10 days.
CSF	LP under aseptic conditions. Collect the CSF in sterile tubes.	Transportation does not need any special media. If one is suspecting bacteria, then transport at ambient temperature as relevant pathogens do not survive well at low temperatures If one is suspecting viruses, then they may be transported at 4 – 8*C. for up to 48 hours.
Faeces	Collect freshly passed stool (8gm) In children, rectal swabs may be used.	Transport in Cary Blair Medium at 4-8*C. Process within 1 – 2 days.
Post mortem samples	Biopsy of relevant tissues Place in formalin for histopathology Place in Transport media for microbiological testing. Place in sterile saline for isolation of viral pathogens	Fixed specimens can be transported at ambient temperatures Specimens in transport media may be transported within 24 hours at ambient temperature. Specimens in sterile saline should be transported at 4-8*C within 48 hours.

***For details, refer to NICD/Manual of laboratory techniques.**

(a) Collection of blood for serological investigations

Performing vene puncture:

- Gloves should be worn. Use sterilized disposable syringes and needles
- For avoiding soiling, a piece of linen with a layer of dressing pad may be placed below the forearm before commencing venepuncture.

- After collecting 5 ml of blood aseptically, it should be carefully transferred from the syringe without squirting in to a sterile screw capped plastic leak proof specimen container. The containers should be labeled before commencement of venepuncture. If the vial has anticoagulants, then a second person wearing gloves should help in shaking the vial for mixing the blood well with the anticoagulants.
- After blood is collected, the tourniquet is removed and the needle is withdrawn. The patient is given a dry sterile cotton swab to press over the site of venepuncture. Elbow may be flexed to keep the cotton swab in place till the blood stops. Any blood spill is carefully wiped with 70% ethanol.
- All the swabs and cotton pieces are placed in plastic bags for disposal. If the outside of the vial is visibly contaminated with blood, it should be cleaned with 10% freshly prepared sodium hypochlorite solution.

(b) Cerebrospinal Fluid (CSF) Specimen Collection

The specimen must be taken by a physician experienced in the procedure. CSF is used in the diagnosis of viral, bacterial, parasitic, and fungal meningitis.

Materials required

- Lumbar puncture tray which includes:
- Sterile materials: gloves, cotton, towels or drapes.
- Local anaesthetic, sterilized needle, syringe.
- Skin disinfectant: 10% povidone iodine or 70% alcohol.
- Two lumbar puncture needles, small bore with stylet (sterilized).
- Six externally threaded sterile screw-cap tubes and tube rack

Method of collection

Only experienced clinician should be involved in the collection of CSF samples. CSF is collected directly into the separate screw-cap sterile tubes. Separate tubes should be collected for bacterial and viral processing.

- Make the patient lie on the bed in left lateral position. Ask the patient to flex the neck (so that the chin touches the chest) hip and the knee joint.
- Using the iliac crest as the reference point, palpate the joint space between the 4th and the 5th lumbar vertebrae and identify the surface anatomy.
- Disinfect the site meticulously with 10% povidone iodine or 70% isopropyl alcohol by swabbing the skin concentrically from the centre of the site outwards. Let the disinfectant evaporate. Do not repalpate the site again.
- Infiltrate the local area with the local anaestheic and wait for 4-5 minutes for the effect to appear before performing lumbar puncture

- Insert the sterile lumbar puncture needle between the 4th and 5th lumbar vertebrae to a depth of 4-5 cm, withdraw the stylet. Fluid flows freely through the needle.
- Between 1 and 2 ml of CSF is collected in each of the 3 tubes, one for culture, one for biochemical analysis and one for cytology.

Note: - Haemorrhagic CSF sample is not recommended for serological testing.

Handling and transportation

- In general, send the specimens to the laboratory and process as soon as possible.
- Transport CSF specimens for bacteriology at ambient temperature, generally without transport media. Never refrigerate the CSF, as many of the bacterial pathogens do not survive well at low temperatures.
- CSF specimens for virology do not need transport medium. They should be transported at 4-8°C.

(c) Stool Sample Collection

Stool specimens are most useful for microbiological diagnosis if collected soon after onset of diarrhoea (for most viruses < 48 hours and for bacteria < 4 days after the onset of illness), and preferably before the initiation of antibiotic therapy. If required, two or three specimens may be collected on consecutive / alternate days. Stool is the preferred specimen for culture of bacterial, viral, and parasitic diarrhoeal pathogens. Rectal swab samples may also be used in case of infants, debilitated patients or while carrying out direct endoscopic visualization of a lesion or any other situation where voided stool sample collection is not feasible. **In general, rectal swabs are not recommended for the isolation of viruses. As far as possible, do not collect stool samples from a bedpan.**

Materials for collection

- Clean, dry, leak-proof screw cap container (which has not been priorly washed with a disinfectant) and tape
- Appropriate bacterial transport media for transport of rectal swabs from infants

Method of collection of stool sample

- Refer to general instructions for collection of stool sample as described above

Method of collection of rectal swab

- Moisten the rectal swab in sterile normal saline
- Introduce the swab inside the anal sphincter and go upto 2-4 cm inside rectum.
- Rotate the swab upto 90 degrees and withdraw the swab.
- Put the swab in any of the transport media like VR fluid / Cary Blair medium by inserting the swab completely into the media.

- Break off the excess wooden portion of swab stick and screw cap the bottle of transport media.
- Store at room temperature till transported to the nearest lab (if cholera is suspected) or else keep it in fridge (4 deg centigrade) if salmonella / shigella is suspected.
- Label the bottle of transport media.

Handling and transport

Two transport media, **V.R. Fluid** (for cholera) and **Cary Blair's** are commonly used for isolation of common bacterial enteropathogens like Salmonella, Shigella, and Esch coli including Vibrios. If the specimens are collected in transport media like **Cary Blair's,** then it should reach nearby laboratory in 2-3 days time and samples can be kept at room temperature. If rotavirus or any other viral etiology is suspected then stool specimen can be kept in fridge (4°C-8°C) - till it is transported to the nearby laboratory.

(d) Respiratory Tract Specimen Collection

Specimens are collected from the upper or lower respiratory tract, depending on the site of infection. Upper respiratory tract pathogens (viral and bacterial) are found in throat and nasopharyngeal specimens. Lower respiratory tract pathogens are found in sputum specimens.

Materials required

- Transport media - bacterial and viral.
- Throat swabs (Dacron and cotton swabs).
- Tongue depressor.
- Nasal speculum.
- 20-50 ml syringe
- Sterile screw-cap test tubes and wide-mouthed clean sterile containers (minimum volume 25ml).

Method of collecting a Throat Swab

- Hold the tongue down with the tongue depressor. Use a strong light source to locate areas of inflammation and exudate in the posterior pharynx and the tonsillar region of the throat behind the uvula.
- Rub the area back and forth with a cotton or Dacron swab.
- Care must be taken to sample the posterior pharyngeal wall at the end to avoid gagging by the patient.
- Withdraw the swab without touching cheeks, teeth or gums and insert into a sterile screw-cap test tube containing appropriate transport medium required.

- Break off the top part of the stick without touching the tube and tighten the screw cap firmly.
- Label the specimen containers.
- Complete the laboratory request form.

Method of collecting per-nasal and post-nasal swabs

- Seat the patient comfortably, tilt the head back and insert the nasal speculum.
- Insert a flexible calcium alginate/Dacron swab through the speculum parallel to the floor of nose without pointing upwards. Alternately, bend the wire and insert it into the throat and move the swab upwards into the nasopharyngeal space.
- Rotate the swab on the nasopharyngeal membrane a few times, remove it carefully and insert it into a screw-cap tube containing transport medium.
- Break off the top part of the stick without touching the tube and tighten the screw cap firmly.
- Label the specimen tube.

Method of collecting nasopharyngeal wash/aspirate

- Have the patient sit with the head tilted slightly backward.
- Flush a plastic catheter or tubing with 2-3 ml of VTM/sterile normal saline.
- Instill 1-1.5 ml of VTM (viral transport medium)/sterile normal saline into one nostril.
- Insert the tubing into the nostril parallel to the palate and aspirate nasopharyngeal secretions.
- Repeat this procedure with the other nostril.
- Collect 1-2 ml in a sterile vial and transport in cold chain at 2-8°C

(e) Collection of Sputum Sample

Materials required

Select a good wide- mouthed sputum container, which is disposable, made of clear thin plastic, unbreakable and leak proof material.

Method of collection

- Instruct the patient to inhale deeply 2-3 times, cough up deeply from the chest and spit in the sputum container by bringing it closer to the mouth.
- Make sure the sputum sample is of good quality. A good sputum sample is thick, purulent and sufficient in amount (at least 2-3 ml).

Handling and transport

If the specimen is collected in the field and can not be immediately processed, it should be transported to the laboratory within 3-4 days of collection. The specimen should be collected in the containers meant for the purpose and lid tightly secured, properly labelled and to be kept away from the sun and heat. These can be placed in a special box, which can withstand leakage of contents, shocks and other conditions incident to ordinary handling practices. These boxes should be kept in the cool conditions and then transported to the laboratory.

(f) Collection of Sputum Sample For Culture

Materials required:

- An ideal container is a wide mouthed, screw cap sterile, Universal bottle, or polypropylene bottle.

Method of collection (as described above):

- It is best to obtain a sputum specimen early in the morning before the patient has taken food, since food particles in smears make them difficult to examine.
- Patients should produce specimens either outside in the open air or away from other people and not in confined spaces such as toilets. A good sputum specimen consists of recently discharged material from the lower respiratory tract, with minimum amounts of oral or nasal material. Ideally, a sputum specimen should have a volume of 3-5ml, although smaller quantities are acceptable if the quality is satisfactory. An early morning specimen, after thorough cleaning of mouth, is preferred.

Handling and Transport:

- **Specimens should** be transported to the laboratory as soon as possible after collection. If delay is unavoidable, the specimens should be refrigerated

(g) Post Mortem Specimen Collection

Need to be collected during outbreak situation when causative agent in not known. Strict precautions, including respiratory protection from aerosolized particles, must be taken when carrying out post-mortem specimen collection in communicable disease outbreaks. Collect the specimens as soon as possible, preferably within 24 hour since viral titres decline while bacteria multiply rapidly after death. Experienced medical personnel may only accomplish thorough post-mortem examinations.

Materials required

- Barrier precautions: double gloves, sterile gown, eye goggles, mask
- For collecting blood and other fluids, refer to corresponding sections for materials
- Aseptic surgical and biopsy instruments for collecting tissue specimens
- Fixatives: saline formalin for histology
- Sterile saline, appropriate viral and bacterial transport media

- Sterile containers, sterile screw cap tubes or vials, glass slides and slide box
- Disinfectant such as household bleach diluted 1:10 in water.

Method of collection

- Use a separate sterile instrument for each tissue specimen from affected sites (several fragments with 1-2 grams of each is sufficient). Smaller, but adequate, specimens may be taken with a biopsy needle.
- Place different tissues in separate sterile containers containing the relevant medium (sterile saline for preparation of tissues for immunofluorescence microscopy; and microbiological transport media for the isolation of bacterial and viral pathogens, fixatives for histopathology).
- Label all containers and tighten the screw caps firmly.
- Blood may be taken from the heart cavities.
- If cerebral malaria is suspected, make several smears from the cerebral cortex on glass slides to detect Plasmodium falciparum. Label the slides and transport in a slide box.

Handling and transportation

- Fixed specimens can be stored and transported at ambient temperature.
- Tissue specimens for isolation of bacterial pathogens can be transported at ambient temperature in transport media for up to 24 hours.
- Transport tissue specimens for isolation of viral pathogens in viral transport medium or sterile saline at 4-8°C for 24-48 hours. For longer periods, freeze and store at -70°C.
- If rabies is suspected and brain samples are collected, freeze unfixed specimens immediately after collection. Formalin-fixed samples are also useful and may be transported at ambient temperature.

(h) Collection of Specimens for Culture

Collection time: In the acute phase of the disease

Specimen to be collected: depends on the Syndrome / presumptive diagnosis: Typhoid/ Cholera: Stool, Blood,

Respiratory specimens (Sputum, Throat swabs, & nasopharyngeal swabs)

Method of collection: refer disease wise.

Disease wise - Sample collection and transportation

Malaria

Laboratory Criteria for Diagnosis

Diagnosis of malaria is done by detecting and identifying malaria parasites microscopically in blood films.

Sample collection for microscopy

Sample to be collected: peripheral finger-prick blood smear i.e. thick and Thin Blood smears

Time of collection

During fever or 2-3 hours after the peak of temperature.

Before the patient receives antimalarial drugs.

Tuberculosis

Laboratory criteria for diagnosis

Demonstration of acid-fast bacilli in at least two of three sputum smears or culture positive for Mycobacterium tuberculosis.

Specimen collection (refer to method for collection as described above)

- A screw capped, wide mouthed, clean, disposable plastic container is used to collect sputum specimens from suspected cases.
- **Three specimens** should be collected for diagnosis as follows.

  One spot specimen when the patient first attends the health service.

  One early morning specimen (preferably the next day)

  One spot specimen when the early morning specimen is being submitted for examination.
- A good sputum sample should be thick, purulent and of sufficient quantity (at least 5ml).

For **Quality assurance,** all positives and 1% of negatives are to be sent to L2 lab for cross checking.

Typhoid

Laboratory criteria for diagnosis:

Serology – Widal/Typhi-dot test positive

Isolation of S.typhi from blood, stool or other clinical specimen.

Sample collection:

Blood and serum

- **Collect aseptically 5ml of blood for serology** (It may be necessary to collect 2 samples at 1 week interval if the first sample is negative and if requested by the L2 Lab). Refer to method for collection of blood as described earlier.
- **Collect aseptically 5ml of blood in BHI broth for culture** (refer to method for collection of blood as described earlier) if it is not possible to collect 2 blood samples, culture can be done from the clot after separation of serum for serology.

Faeces for culture

On some occasions it may be necessary to collect stool samples. Request the specimen from the patient. The patient must be provided with a wide necked container to pass the faeces. The container must not contain any disinfectant. The patient must be instructed not to contaminate the faeces with the urine.

Transfer a portion of specimen using cotton wool swab, into Cary Blair medium and transport it to the L2 lab within 48 hours. Cold chain is not required.

Cholera

Laboratory criteria for diagnosis:

Isolation of Vibrio cholera O1 or O139 from stools in any patient with diarrhoea

Collection of sample: Faeces for V. Cholera isolation

- Follow general instructions for stool sample collection (as described above)
- Transfer a portion of specimen to a cotton wool swab; insert it in Cary Blair's transport medium. (The medium can be obtained from L2 lab every three months)
- If stool specimen could not be collected take a rectal swab and insert it in the above solution.
- Complete the lab request form.

Leptospirosis

Laboratory criteria for diagnosis

- Isolation from blood or other clinical materials by culture
- Positive serology, preferably Microscopic Agglutination Test (MAT) using a panel of *Leptospira* strains

Collection of samples

Blood: During first week of illness collect

- 2 ml of venous blood with anticoagulant (Heparin or EDTA) using sterile precautions, for dark-field microscopy (method of collection of blood as described earlier)

- 3 ml of blood for serology using a sterile, dry syringe to avoid haemolysis (method of collection of blood as described earlier).

Urine: Urine should be collected after second week of illness and transported immediately in sterile container (acidic urine is inhibitory to leptospires).

(Second serum sample to be collected after 5 days if required, for demonstrating rising titre or sero conversion).

Handling and Transport

- Store serum in refrigerator. If there is delay in transportation, store the serum in deep freezer at –20 ° C.
- Transport serum as quickly as possible, within 24 hours to L2 lab.

Measles

Laboratory criteria for diagnosis:

☞ Presence of measles virus specific IgM antibodies

☞ At least fourfold increase in antibody titre in paired samples

☞ Isolation of measles virus

7.8 Specimen Collection, Processing and Transportation

Blood sample for serology:

An acute phase serum specimen (3-5ml of whole blood) should be taken soon after onset of clinical symptoms but not later than 7 days.

Samples for virus isolation:

(i) Urine Specimens:

Urine should be collected within 5 days of rash onset (1-3days best) First morning mid stream voided specimens are preferable. Urine should be collected in sterile wide mouthed containers.

NOTE: Do not freeze the urine specimen. The entire specimen should be stored at 4°C or (Icepack) till transported to the Virus Isolation lab - L3 lab.

(ii) Respiratory Specimens (nasopharyngeal swab, pharyngeal and throat swab):

The specimens should be collected within 5days after the onset of rash. Follow general instruction for swab collection (as described earlier). The tip of the swab is put into a vial containing 1.5-2ml of transport medium and the applicator stick is broken off.

The sterile cotton swabs can be prepared in the L1 / L2 labs.

The VTM will be supplied by the L3 lab on request

Storage of Samples:

The samples should be stored in the refrigerator. If there is a delay in transport, the serum and nasopharyngeal swab in VTM should be stored in the -20 deep freezer. The urine sample should not be frozen.

Transportation:

- ☞ All samples should be transported maintaining cold chain.
- ☞ The carrier should be marked as 'Potentially Hazardous'.
- ☞ If any delay in transportation, samples should be stored at 4°C
- ☞ Transport the specimen always 'in person' to L3 lab

Dengue

Laboratory criteria for diagnosis:

Any one or more of the following:

1. Isolation of dengue virus from serum, plasma, leucocytes, or autopsy samples
2. Demonstration of Dengue virus specific IgM antibodies or fourfold or more rise in reciprocal IgG antibody titre
3. Demonstration of dengue antigen in autopsy tissue by Immunochemistry or immunoflourescence or in serum samples by EIA
4. Detection of viral genomic sequences in autopsy tissue, serum or CSF by PCR

Sample Collection

Sl. No.	Sample	Period of Collection	Method of Collection	Qty	Storage for 24 to 48 hrs	Trans	Trans Temp
1	Serum	5 days after onset	Collect Intravenous blood	3 – 5 ml	+4°C	L2	Cold chain
2	Plasma (Citrated blood)	Within 5 days of onset	Collect Intravenous blood in a citrated tube	3 – 5 ml		L3	
3	CSF	Within 5 days of onset	Collect CSF by lumbar puncture in a sterile vial	2 ml			
4	Autopsy (brain, lung, liver)	In the event of death	Collect in a sterile container	Small quantity			

*** 5 to 10 representative samples should be sent to L3 Lab for virus isolation and serotyping in case of outbreaks.**

Japanese Encephalitis: (Fever with Altered Consciousness)

Laboratory criteria for diagnosis

Laboratory diagnosis of JE is done by the following methods:

- Demonstration of JE virus specific IgM antibodies
- Detection/isolation of antigen/virus
- Demonstration of viral antigen in the autopsied brain tissue by the fluorescent antibody test

Presumptive

Detection of an acute phase anti-viral antibody response through one of the following:

Elevated and stable serum antibody titres to JE virus through haemagglutination- inhibition or IgM antibody to the virus in serum/CSF by ELISA

Confirmed

Detection of JE virus, antigen, or genome in tissue, blood or other body fluids by immunohistochemistry or immunoflourescence or PCR.

Sample Collection

Sl. No.	Sample	Period of Collection	Method of Collection	Qty	Storage for 24 to 48 hrs	Trans	Trans Temp
1	Serum/ plasma	Within 6 days of onset	Collect Intravenous blood as per the guidelines	3 – 5 ml	+4°C	L3	Cold chain
2	CSF	Within 6 days of onset	Collect CSF by lumbar puncture in a sterile vial	2 ml			
3	Autopsy brain Tissue	In the event of death	Collect in a sterile container	Small qty			

Plague

Plague is a zoonotic disease caused by a bacterium ***Yersinia pestis***, transmitted between rodents and human beings through infected rodent fleabites.

Suspected Plague:

1. Clinical symptoms that are compatible with plague, e.g. fever sepsis syndrome, lymphadenopathy and/or acute pneumonitis in a person who resides in or recently traveled to a plague –endemic area
2. If gram negative and/or bipolar –staining coccobacilli are seen on a smear taken from affected tissues, e.g.,
   - Bubo (bubonic plague)

- Blood (septicemia plague)
- Tracheal/lung aspirate (pneumonic plague)

Presumptive:

- Y.pestis F1 antigen detected in clinical materials by direct fluorescent antibody testing, or by other standardized antigen detection method or
- Isolate from a clinical specimen or
- A single serum specimen is found positive for diagnostic levels of antibodies to Y.pestis F1 antigen, not explainable on the basis of prior infection or immunization

Confirmed:

- Isolate identified as Y.pestis by phage lysis of cultures; or
- A significant (equal or more than 4-fold) change in antibody titre to the F1 antigen in paired serum specimens. or
- PCR positive

Clinical samples to be collected

- Blood, Serum, bubo aspirate and sputum.
- Organ for culture & smears - Lungs, Liver and spleen of Rodents
- The clinical samples are to be collected during the acute phase of illness. 8 to 10 ml of blood to be collected for blood culture and serology.
- Storage: All clinical specimens should be stored at 4°C.

Transport of Clinical Samples

The methanol fixed smears are to be sent to the L2 in a slide mailer. The clinical samples and rodent tissue samples for culture, serology and PCR are to be transported to L3 laboratory maintaining cold chain.

Collection Procedure - The kit for collection of sample to be brought by Surveillance team (Kit containing the following: 10-20 ml syringe, 18 –20 gauze needle, petridish, sterile swab stick, clean dry vials, sterile normal saline, Cary Blair Transport medium)

Collection of Bubo aspirate

- Sterile the surface of the bubo with tincture iodine
- Draw a few ml of sterile physiological saline in a 10 –20 ml syringe fitted with 18 or 19 gauge needle
- Puncture the bubo and apply suction. If bubo fluid could not be aspirated, inject saline into the bubo and aspirate again.
- Make smears with the bubo aspirate

- Transfer the bubo aspirate in the Cary Blair Transport Medium for transportation to the laboratory.

Sputum, Throat swab, and blood follow universal procedure and precautions as described earlier

- Ask the patient to expectorate into a sterile, wide mouthed container such as petridish

Precautions in handling specimens

As these specimens are known or thought likely to contain infectious substances, the following precautions should be applied

- Strict aseptic technique (gowns, gloves, masks)
- Wash hands before and after the collection of material
- Place the specimen aseptically in an appropriate sterile container
- Tightly close the container
- Label and date the container

Packaging/Transportation of Specimen:

In accordance with currently accepted bio-safety norms, **Y.pestis is listed under Bio-safety 2 level.**

The following practices, should be observed

- A watertight primary receptacle
- A watertight secondary recetable
- An absorbent material, which must be placed between the primary receptacle and the secondary packaging. The absorbing material must be adequate to absorb the entire contents of all primary receptacles.
- Itemized list of contents must be enclosed
- Outside package must be marked with identification of the infectious substance, volume of contents, name, and telephone number of sender

Hepatitis

Hepatitis surveillance is sentinel based. However, while investigating a case of syndrome of jaundice, hepatitis needs to be investigated on a routine basis also.

Laboratory criteria for diagnosis:

Hepatitis A: IgM anti HAV positive

Hepatitis B: Positive for HbsAg or IgM anti-HBc

Hepatitis C: Positive for anti-HCV

Hepatitis D: Positive for HbsAg or IgM anti-HBc Plus anti-HDV

Hepatitis E: Positive for anti-HEV

L1 lab: Collect serum samples (as described earlier) (Using bio-safety guidelines) and transport to L2 labs in cold chain.

L2 laboratory

- Perform Rapid tests for
  - HbS Ag, - Latex agglutination and Dip stick ELISA
  - Hepatitis C - Immunochromatic test
- Transport to L3 under cold chain for Hepatiits A, D and E serology

Testing of Water Samples

In the laboratory there are two simple procedures of testing water samples for faecal contamination:

1. **Most Probable Number (MPN) method for coliform bacteria –** using the multiple tube fermentation technique in this method the MPN of total coliform bacteria, faecal coliform bacteria (or the thermo tolerant coliforms) present in the water sample is determined, along with the presence/absence of Escherichia coli.

2. **H2S-Strip method:** This is a simple, reliable and easy-to-perform (by even untrained personnel), Presence/Absence test for bacteriological quality Which works on the principle that there is a close correlation between faecal contamination and the presence of hydrogen sulphide (H_2S) producing bacteria and, that faecal pollution of water can be deduced by demonstration of H_2S production. It has been claimed, by various workers, that the H_2S-strip method shows >90% agreement with the conventional MPN test described above.

General instructions for collection, storage and transport of water samples for bacteriological examination:

1 Care must be taken to ensure that samples are representative of the water being examined.

2 Care must be taken to see that no accidental contamination occurs during sampling: The sample collector must wash his/her hands well using carbolic soap, just prior to collection of samples.

3 Test the water samples as soon as possible after collection. If delay of more than 3 hours is expected, transport samples to laboratory under cold-chain conditions.

4 A volume of water sample adequate to carry out all the tests should be collected: generally, 200-250 ml of water sample should be collected.

5 Samples for bacteriological examination shall be collected in clean, sterilized, narrow mouthed neutral borosilicate glass bottles or Autoclavable plastic (poly propylene) bottles with screw cap lids.

6 Collection of samples from taps: Available in the manual available with the laboratory.

Materials for collection

- Glass bottles with securely fitting stoppers or caps having an overhanging rim. Both the bottle and the cap/stopper should be adequately sterilized. The bottle should be able to hold at least 200ml of water. Alternatively, autoclavable plastic bottles with a tight screw capped lid may be used.
- Sodium thiosulphate (0.5ml of 10% solution or a small crystal), in case collecting sample from chlorinated source of water.

Method of collection

Sampling from a tap or a pump or a pump outlet

- Remove from the tap any attachments that may cause splashing and using a clean cloth, wipe the outlet removing any dirt.
- Turn on the tap at maximum flow rate and let the water flow for 1-2 minutes. Close the tap
- Sterilize the tap for a minute with flame: a gas burner or a cigarette lighter may be used.
- Carefully turn on the tap and allow the water to flow for 1-2 minutes at a medium flow rate.
- Open a sterilized bottle with its protective cover on. While holding the cap and the protective cover face downwards (so as to prevent entry of dust that might carry microorganisms), immediately hold the bottle under the water jet, and fill.

  A small airspace should be left to facilitate shaking at the time of inoculation prior to analysis.
- Place the stopper in the bottle or screw on the cap and fix the brown paper protective cap in place with the string.

Sampling from a watercourse or reservoir

- Holding the bottle by the lower part, submerge it to a depth of about 20cm; with the mouth facing slightly upwards; if there is a current, the bottle mouth should face towards the current.

Sampling from dug wells or similar sources

- With a piece of string attach a stone of suitable size to the sampling bottle.
- Take a 20m length of clean string rolled around a stick and tie onto the bottle string. Open the bottle as described earlier.
- Lower the bottle, weighted down by the stone, into the well, unwinding the string slowly. Do not allow the bottle to touch the sides of the well.

- Immerse the bottle completely in the water and lower it down to the bottom of the well.
- Once the bottle is judged to be filled, rewind the string round the stick to bring up the bottle. If the bottle is completely full, discard some water to provide airspace.
- Stopper or cap the bottle as described earlier.

Handling and transport

- Test the water sample within 3 hours of collection during which time it can be kept at ambient temperature. If more delay is expected, pack the water sample in ice for transport to the laboratory.
- The refrigerated sample should be tested within 24 hours.

SUMMARY OF SAMPLE COLLECTION / TRANSPORTATION/ LAB INVOLVED IN TESTING

Disease	Specimen collected/by whom	Quantity	Condition for collection	Storage temp	Trans temp	Test done at
Malaria	Peripheral blood smear for microscopy by PHN / lab asst	Thick and thin smear in single slide	Clean slides	Room temp	Room temp	L1
TB	Sputum for microscopy	5 – 10ml	Clean containers	RT	RT	L1
	Sputum for culture by VHN / lab	5 – 10ml	Sterile	RT	RT	L3
Typhoid	Blood for serology	5 ml	Sterile	+4°C	RT	L1 (Typhidot)
	Blood for Serology, culture & Sensitivity by MO / Tech	5 ml in Cary Blair medium	Sterile	+4°C	RT	L2 (Widal)
Cholera	Stools for culture by VHN		Clean containers	+4°C	RT	L2
Leptospirosis	Blood for serology by MO / tech	5 ml	Sterile	+4°C	RT	L2
Polio	Stool for culture by VHN	2samples 24 hrs apart	Clean	+4°C	Cold chain	L3

Disease	Specimen collected / by whom	Quantity	Condition for collection	Storage temp	Trans temp	Test done at
Measles	Blood for serology by MO / Tech	5 m from 5 to 10 cases	Sterile	+4°C > 24hrs at –20°C	Cold chain	L3
	Urine for culture by VHN	15 ml	Sterile	+4°C	Cold chain	L3
	Naso pharyngeal swab by Tech	In VTM	Sterile	+4°C > 24hrs at –20°C	Cold chain	L3
Dengue	Blood for serology by MO	5 ml	Sterile	+4°C > 24hrs at –20°C	Cold chain	L2- rapid test L3 - ELISA (IgM)
	Blood for culture by MO	5 ml	Sterile	+4°C > 24hrs at –20°C	Cold chain	L3
JE	Blood for serology by MO	5 ml	Sterile	+4°C > 24hrs at –20°C	Cold chain	L3 - ELISA
Hepatitis	Blood for serology by MO	5 ml	Sterile	+4°C > 24hrs at –20°C	Cold chain	L3 - ELISA
Water quality	Water samples by tech	50 – 100 ml from different sources	Sterile	RT	RT	L1 – H2S L2 MPN
Plague	Blood for smear by MO	Drop	Clean slide	RT	RT	L2

7.9 Recording and Reporting

Inputs from laboratory in the form of accurate and timely data are the most important activities in disease surveillance, without which, all the efforts in building the system would go waste. The laboratory data management includes:

- Recording details of specimens received
- Record of tracking of samples
- Recording results of tests performed
- Analysis and interpretation of tests
- Timely and accurate communication of results

7.10 Recording of Data:

Information to be recorded on each specimen/ accompanied with each specimen:

- Name, age, sex,
- Address in detail,
- From whom referred
- Syndromic diagnosis
- Date of onset of illness

- Nature of sample, date of collection, date of receipt, condition of sample,
- Investigation requested
- Whether convalescent specimen or not

From the lab request form, relevant information is written in the lab register after assigning a laboratory number.

SAMPLE LAB REGISTER

Date:

ID no	Name and address of patient	Age	Sex	Provisional Diagnosis	Lab tests ordered	Lab results	Date sent to L2	Result from L2	Date of result
1									
2									
3									

On every Monday, the laboratory is expected to send a weekly report on the tests performed along with results in the prescribed form to the District Surveillance Officer. Even if no test was performed, a nil report is a must to ensure regularity in reporting.

Diseases of public health importance like Cholera, Dengue Fever, Diphtheria, Japanese Encephalitis, Leptospirosis, Plague, Whooping Cough, etc must be reported to the District Health Authorities immediately.

Specimen referral form

Referring lab:

Lab No:

Name and address of patient

Age

Sex

Provisional Diagnosis

Lab tests ordered

Date of Collection

Date of dispatch

Remarks

Lab I/**c**