

# CLINICAL SERVICE LABORATORY : SAMPLE COLLECTION METHODS

## **1.0 PURPOSE & SCOPE**

All laboratory procedures for clinical service laboratory testing are performed on different types of specimens like sputum, pus, urine, blood; serum or plasma, tissues, CSF and different body fluids. The quality and quantity of a specimen is of paramount importance for getting accurate test results and for the interpretation of these results to be scientifically sound. This document will describe in detail this procedures for specimen collection, their storage & proper transport. It is essential that the staff is aware of proper timings, collection techniques, container types, and quantity of specimen, special requirements and proper care and preparation of the patient. Specimens must also be transported within predefined time limits to the microbiology laboratory under proper conditions for processing. Proper storage of collected specimens must be done in case of delay in transportation. This SOP details the procedure for collection, their storage, transportation & rejection criteria.

This SOP is applicable to all clinical & laboratory staff dealing with sample collection of Clinical Service Laboratory of B.J.Govt. Medical College, Pune and is available to all clinic staff at the clinic sites. It should not be removed from the sites or copied without prior permission from management.

## **2.0 Responsibilities**

- 2.1 It is the Responsibility of the authorized doctor , nurses and technician to collect, transport & suitably store the specimen if required.
- 2.2 It is the responsibility of the authorized doctor to counsel the patient for HIV testing,before collecting the sample
- 2.3 It is the responsibility of the laboratory staff of ICTC laboratory and CD4/CD8 laboratory,to collect as well as receive samples, and report the results.
- 2.4 It is the Responsibility of the Laboratory technician in collection center in microbiology department to check for the rejection criteria, receive and send the sample further for testing to the respective sections.

## **3.1 PROCEDURE FOR SERUM/PLASMA SAMPLES:**

**3.1.1** Whole blood is collected in red top vacuutainer for serum

**3.1.2** Whole blood is collected in grey top vacuutainer for plasma.

### **3.1.2 Material required:**

- Skin disinfection: Spirit(disinfecting agent),swabs, gauze pads ,cotton
- Disposable latex gloves
- Tourniquet, plain vacuutainer tubes (red tops) fluoride vacuutainer (grey), needles, needle holder & tube holder, or disposable syringes & needles.
- Labels & hard ball point pen/marker
- Puncture resistant discard jar containing freshly prepared 1% sodium hypochlorite solution
- Blotting Papers
- Scalps

### **3.1.3 Method of Collection**

- Lab requisition form is reviewed for completeness & identity of patient.
- Label the vacuutainer with patents details like PID No, Date & time of collection and collectors initial.
- Use a clean set of gloves.
- Place a tourniquet above the venipuncture site. Try and minimize the time the tourniquet is on to 1-2 minutes.

- Palpate and locate the vein. If too much time has gone by after locating the vein release tourniquet and wait a few minutes before attempting venipuncture.
- It is critical to disinfect the venipuncture site meticulously with Spirit by swabbing the skin concentrically from the centre of the venipuncture site outwards. Let the disinfectant evaporate. Do not re-palpate the vein again. Perform venipuncture.
- If you need second attempt of venipuncture use other arm.
- If withdrawing using vacuum systems, withdraw the desired amount of blood directly into each collection tube. For adult patients withdraw 3-5 ml of blood & pediatric patients 2-3 ml should be withdrawn.
- After filling all tubes or syringes remove the needle from venipuncture site.
- Remove the tourniquet. Apply pressure with gauze or cotton to site until bleeding stops. Apply bandage if desired.
- Discard needle directly into the sharps disposal container. Do not recap used sharps.
- After sample collection keep the vacutainer tube in the rack.
- Enter the sample details in sample receiving register maintained in ICTC
- As the laboratory is located in the same campus the specimens will be transported to the lab at room temperature in a properly labeled transport container by hand. Samples are transported to the lab by attendant or technicians themselves.

#### **3.1.4. Certain areas are to be avoided when choosing a venipuncture site:**

- Extensive scars from burns and surgery - it is difficult to puncture the scar tissue and obtain a specimen.
- The upper extremity on the side of a previous mastectomy - test results may be affected because of lymphedema.
- Hematoma - may cause erroneous test results. If another site is not available, collect the specimen distal to the hematoma.
- Intravenous therapy (IV) - fluid may dilute the specimen, so collect from the opposite arm if possible.
- Edematous extremities - tissue fluid accumulation alters test results.

#### **3.1.5 Special Precautions:**

- *Vasovagal response* can cause dizziness, sweating, coldness of skin, numbness and tingling of hands and feet, nausea, vomiting, possible visual disturbance, fainting and injury fall from fainting.
- If during venipuncture the patient complains of dizziness, nausea or any of the above signs are noticed release the tourniquet and remove the needle immediately.
- The patient must not be allowed to leave the collection place. Patient is allowed to relax, leg are raised. Give 25% Dextrose. A clinic nurse or physician will be notified to examine the patient.

#### **3.1.6 Safe handling & Disposal of sharp at collection center:**

- Care should be taken to avoid auto inoculation by used sharp
- Discard all chipped or cracked glassware in appropriate containers
- Do not manipulate, bend, break, recap or remove the used disposable needle from the syringe.
- Do not pass used sharp directly from one person to another
- Refer SOP: BJMC/HIV/Gen-SOP/1.6 (Biomedical waste management) & HIV/BJMC/MSD/02 (Safety manual)

## **3.2 TROUBLESHOOTING**

### **3.2.1 To Prevent a haematoma:**

That is noticeable bruising at the site of venipuncture

- Puncture only the uppermost wall of the vein

- Remove the tourniquet before removing the needle
- Use the major superficial veins
- Make sure the needle fully penetrates the upper most wall of the vein.( partial penetration may allow blood to leak into the soft tissue surrounding the vein by way of the needle bevel)

### 3.2.2 **Haemoconcentration:**

An increased concentration of larger molecules and formed elements in the blood due to several factors:

- Prolonged tourniquet application(no more than 2-3 min)
- Massaging or squeezing the site
- Sclerosed or occluded veins

### 3.2.3 **Haemolysis**

Haemolysis may occur through mechanical means or due to some disease or physical disorder. Though auto-haemolysis is a possibility, the most likely reason is mechanical and is ultimately the result of incorrect blood drawing or specimen processing techniques. Haemolysis can adversely affect many laboratory results. Therefore it is critical to use proper techniques

- When drawing with a syringe, **DO NOT** draw back on the plunger forcefully. A nice even slow draw is preferred
- Use the appropriately size needed for the vein.
- If a syringe is used and blood is injected into an evacuated tube, use a 21 gauge needle and **NEVER** force the blood into the tube by pushing the plunger. Allow the vacuum to draw the blood from the syringe.
- Alternatively, remove the stopper from the sample tube, remove and dispose of the needle then slowly force the blood out of the syringe by pushing on the plunger. Allow the blood to flow down the side of the tube
- **DO NOT** mix vigorously or shake the specimens

### 3.3 **SPECIMEN RECEIVING:**

All specimens received in the laboratory will maintain the chain of custody from the collection sites. This involves the proper identification of specimens with its lab requisition form (tracking sheet) and the proper review of all information.

- Review the received specimens with its laboratory requisition form (For HIV Testing).
- Verify the specimen information found on the lab requisition form/HMIS matches the information found on the specimen.
- The following will be reviewed and verified.
  - Patient ID # like PID ir MRD No
  - Specimen type or source
  - Condition of the specimen
  - Type and quantity of collection tubes or container,
  - Information on collection labels or container
  - Collection date & time
  - Technician Initials.
- After verifying all required information on the lab requisition form & sample rejection criteria (section 3.4) will be looked for to accept or reject the samples.
- If the sample is rejected the concern clinician or the patient himself is contacted and requested to re-collect the sample.
- Action to be taken on rejected samples either in ICTC or in clinical service laboratories
  - If the sample is rejected then mention it on the comment section of the requisition form. Mention the reason for rejection and request re-collection of sample.
  - Enter the details of rejected sample in Rejection log book
  - For HIV testing :** The following information will be written in the Laboratory register provided by MSACS.
    - Site ID

- PID No
- Sample No.
- Date of performing test
- Details of HIV test kits used & Results obtained
- Comments

### **3.4 Rejection criteria:**

- Samples brought by unauthorized person
- Sample not properly transported ( packaging, temperature, delayed transit)
- Sample not labeled/label illegible
- Label not matching with that of lab requisition form
- Sample not accompanying of lab requisition form
- Sample not accompanying proper/complete lab requisition form
- Sample : insufficient quantity (to test/to repeat test/ back up)
- Sample leakage
- Haemolysed sample
- Lipaemic sample
- Sample turbid( contaminated)
- Sample coming/collected beyond stipulated period
- Sample coming beyond working hours(at odd hours) to the SRL
- Sample collected in improper container

### **4.0 SPECIMEN RECEIVING:**

All specimens received in the Microbiology laboratory (timing 10-1pm) will be received at the central collection centre. Following this till 5 Pm samples can be submitted in the respective sections.

- Verify that the requisition for the test/tests are made on the HMIS.
- Transfer the sample to the serology laboratory at 1pm
- After verifying HMIS entry , all tests required for the same are noted
- Label the test tube with laboratory numbers for various tests.
- After assessing sample rejection criteria (3.5 above) sample will be accepted or rejected .
- If the sample is rejected the concerned clinician/ nurse is contacted. The sample is recollected.

### **5.0 GENERAL INSTRUCTIONS AND BIOSAFETY PRECAUTIONS FOR SAMPLES TO BE COLLECTED FOR CULTURE.**

- All samples should be considered potentially infectious and necessary precautions should be taken.
- All specimens collected for culture should be collected before starting any antibiotics or before the next dose of antibiotic is due.
- Mention if patient is receiving any antimicrobials and if it is a follow-up sample.
- Apply strict aseptic techniques for collection .
- Wash hands before and after the collection
- All specimens should be collected in appropriate sterile containers which are available in the collection centre from 10am to 5 pm and after that from the resident microbiologist on call in the CCL.(Central clinical laboratory).
- Samples are received through the day 10am to 5 pm in the Microbiology laboratory.
- After 5pm sample should be sent to resident microbiologist on call in the CCL.(Central clinical laboratory).who will process the sample.
- Ensure that the outside of the specimen container is clean.

- Tightly close the container.
- Appropriately label and date the container and complete the requisition form.
- Arrange for immediate transportation to the laboratory. (within 2 hrs)if a delay is expected take precautions as mentioned with each sample type

## **6.0 INDIVIDUAL SPECIMEN COLLECTION AND TRANSPORT METHODS**

### **6.1 Abscess-Superficial/wound infection**

#### **Container, Specification/Materials required:**

Two Sterile swabs in sterile test tube

#### **Method of collection :**

Wipe area with sterile saline / 70% alcohol. Collect fresh pus that oozes out or apply firm pressure to release pus and collect with sterile swab which should be placed in a sterile tube. If no pus is oozing , swab from under the scab / from edge of wound.

#### **Transport:**

Swabs in sterile test tubes should be transported to the laboratory as early possible (within a few hours) .

#### **Rejection Criteria:**

Dry swabs

Sample without appropriate label.

Requisition not made on HMIS/form

### **6.2 Abscess-**

#### **Container, Specification/Materials required:**

Sterile test-tube /RCMM

#### **Method of collection :**

Wipe area with sterile saline / 70% alcohol. Aspirate the pus with the sterile needle and syringe and transfer to a sterile tube immediately.

**For anaerobic processing. ONLY.** Aspirate material from depths of lesion with a 5-10 ml syringe inoculate in RCMM if RCMM not available impale needle into a sterile rubber bung. Should reach lab within 30 min of collection.

**For gas gangrene** collect swab from the depth of the wound ,prepare two smears and collect sample in RCMM.

#### **Transport:**

The sample - should be transported to the laboratory as early possible 2-3hrs

#### **Rejection Criteria:**

Dried pus

Sample without appropriate label.

Requisition not made on HMIS/form

### **6.3. Granules from suspected fungal lesion -**

#### **Container, Specification/Materials required:**

Sterile blade/ needle and sterile test-tube

#### **Method of collection :**

Locate an area where the granule is likely to break out (Patients can usually tell you ) Wipe the area with sterile saline / 70% alcohol. Make an incision and coax the granule out with a sterile blade. Transfer to a sterile tube immediately. Sometimes if the wound has been dressed, granules may be seen on the dressing, these should be lifted with a sterile blade/needle and transferred to a sterile test tube.

**Transport:** In sterile test tube should be transported to the laboratory as early possible

**Rejection Criteria:** Sample without appropriate label. Requisition not made on HMIS/Form

#### 6.4. Blood for culture

##### **Container, Specification/Materials required:**

In house Blood culture bottles/Bactec blood culture bottles (separate bottles are available for paediatrics patients as well as for aerobic/ anaerobic/ tuberculosis/ fungal culture. Please collect the appropriate bottle )

Skin disinfection: Spirit(disinfecting agent),swabs, gauze pads ,cotton

Sterile latex gloves

Tourniquet, Labels & hard ball point pen/marker .Sterile needle and syringe.

##### **Method of collection :**

Place the tourniquet above the elbow joint and tighten it, asking patient to clench his fist.

Palpate and locate the vein and clean once with spirit using circular swabbing in increasing circles from within outwards. Release the tourniquet

Keep the labelled (on which time of collection is mentioned) blood culture bottle at hand. Wipe the cap with spirit.

Wash your hands with soap and water and wear sterile gloves .

Take relatives help to tighten tourniquet.

Clean intended skin site with povidone iodine, followed by methylated spirit / 70% alcohol again .

Allow to dry .

Withdraw the desired amount of blood (minimum 5ml-7ml) directly into blood culture bottle. Shake gently .

Remove the tourniquet ,apply pressure with cotton swab dipped in spirit at the site until bleeding stops.

##### **Transport: T**

The blood culture bottle should be transported to the laboratory as early possible. If a delay is expected keep at room temperature DO NOT refrigerate .

##### **Special Instructions:**

Allow blood culture medium to warm to ambient temp before inoculating.

Collect blood culture at the first instance that septicemia is suspected preferably 2 sets from either arm.

For BM inoculate culture medium before making smears. Collect 8-10 ml for adults and 1-5 ml for children. For neonates, 1 ml blood is sufficient.

**Rejection Criteria:** Sample without appropriate label.

Requisition not made on HMIS/form

#### 6.5. Body fluids (Ascitic / pleural / pericardial / peritoneal)

##### **Container, Specification/Materials required:**

Sterile tube /Blood culture bottle

Labels & hard ball point pen/marker

Appropriate tray which includes :

Sterile materials : gloves, cotton, towels or drapes.

Local anaesthetic, sterilized needle, syringe.

Skin disinfectants : 10% povidone iodine or 70% alcohol

Two sterile needles and syringes with /without stylet (sterilized)

##### **Method of collection :**

Locate the appropriate area and clean the skin once with spirit using circular swabbing in increasing circles from within outwards.

Keep the labelled sterile tube handy .

Wash your hands with soap and water and wear sterile gloves .

Cover the area with a sterile drape.

Clean intended skin site with povidone iodine, followed by methylated spirit / 70% alcohol again . Allow to dry .Collect the sample (8-10ml) from appropriate site with full aseptic precautions .

Transfer to sterile tube (ensure the plug does not get contaminated)

**Transport:**

The sterile tube should be transported to the laboratory as early possible.  
If a delay is expected keep at room temperature. DO NOT refrigerate .

**Rejection Criteria:**

Sample without appropriate label. Requisition not made on HMIS/Form.

**6.6.Cerebrospinal Fluid (CSF)**

**Container, Specification/Materials required:** Sterile tube/blood culture bottle

Labels & hard ball point pen/marker

Lumbar puncture tray which includes :

Sterile materials : gloves, cotton, towels or drapes.

Local anaesthetic, sterilized needle, syringe.

Skin disinfectants : 10% providone iodine or 70% alcohol

Two lumbar puncture needles, small bore with stylet (sterilized)

**Method of collection :**

Locate the L4 L5 Area and clean the skin once with spirit using circular swabbing in increasing circles from within outwards.

Keep the labelled sterile tube handy .

Wash your hands with soap and water and wear sterile gloves .

Cover the area with a sterile drape.

Clean intended skin site with povidone iodine, followed by methylated spirit / 70% alcohol again . Allow to dry .

Collect the sample directly into the sterile tube first .

**Transport:**

The sterile tube should be transported to the laboratory as early possible.  
If a delay is expected keep at room temperature. DO NOT refrigerate .

**Special Instructions:**

Please mention all the tests required (bacterial/fungal/AFB )culture/VDRL.

**Rejection Criteria:**

Sample without appropriate label.

Requisition not made on HMIS/Form.

**6.7.Conjunctival Swab**

**Container, Specification/Materials required:**

2 Sterile swab moistened with sterile saline with sterile test tube

**Method of collection :**

Collect taking care to reduce blinking. If collected from both eyes label correctly .

**Transport:**

Swabs in sterile test tubes should be transported to the laboratory as early possible (within a few hours) .

**Rejection Criteria:**

Dry swabs .Sample without appropriate label.

Requisition not made on HMIS/Form

**6.8. Corneal Scrapping Container, Specification/Materials required:**

Slides and sterile test tubes/Collect appropriate media (Blood Agar/Sabourauds Dextrose Agar)

**Method of collection :**

After applying eye speculum with a sterile blade take a scrapping and inoculate directly on the media or put in the sterile tube .Make smears from another scraping. Air dry the smears before wrapping in paper.

**Transport:**

Swabs in sterile test tubes should be transported to the laboratory as early possible (within a few hours) .  
Smears to be transported wrapped in clean paper.

**Rejection Criteria:**

Sample without appropriate label.

Requisition not made on HMIS

**6.9. Faeces Container Specification/Materials required:**

Sterile wide mouth container

**Method of collection :**

Ask the patient to collect faeces in the container without allowing urine to mix with it. Avoid soiling the outside of the container.

**Transport:**

Transport to the laboratory as soon as possible.

**Special Instructions:** Mention the test required eg hanging drop preparation/ culture/parasitology

If cholera is suspected ask for alkaline peptone water .

**Rejection Criteria:**

Sample without appropriate label.

Requisition not made on HMIS

**6.10. Faeces –Rectal Swab**

**Container Specification/Materials required:** Ask for appropriate transport medium (Cary Blair/  
Alkaline peptone water)

**Method of collection :** Moisten a cotton swab with sterile saline.

Insert it inside the anal sphincter and go up to 2-4 cm inside the rectum.

Gently rotate up to 90 degrees, so that faeces covers the swab. Withdraw the swab

Place it in transport medium, break off the top portion of swab stick and discard

Label the specimen

**Transport:**

Transport to the laboratory as soon as possible .

**Rejection Criteria:**

Sample without appropriate label.

Requisition not made on HMIS/Form.

**6.11 Gastric aspirate:**

This is usually collected in infants and children who cannot expectorate sputum. It is an indoor procedure ,patient should be admitted a day prior to the procedure

**Container Specification/Materials required:**

**N95 masks, gown Cap,** Infant Ryle's tube, sterile 20 ml syringe, sterile distilled water 50ml

Sterile wide mouth container with sodium bicarbonate

**Method of collection :**

**Preparation the Night before the Procedure**

The child is preferably hospitalized overnight in order to assure adherence with schedule of fasting before and after placement of NGT

Nothing per mouth after 1:00 AM

Maintain child in decubitus (lying down) position at all times (avoid sitting and standing positions to prevent emptying of stomach).

Do not administer food or medications by mouth until the NGT is removed.

Encourage parents, caregivers and healthcare workers to keep the child asleep by minimizing noise and disruption, because on waking: Peristalsis is activated and stomach empties (including the swallowed sputum) Standing or lifting the child may cause the stomach to be emptied by gravity

## **Insertion of the NGT**

Extend the patient's head slightly back, avoiding hyperextension, to facilitate the entry of the NGT into the esophagus.

Lubricate the NGT with lidocaine

Manipulation of the portion of the NGT that is distal to the nostril should be performed with aseptic technique using sterile gloves

Insert the NGT through the larger nasal passageway.

To facilitate the passage of the NGT in older children, instruct the child to “swallow” the tube.

The NGT may stop advancing for one of the following reasons:

Curling into the oral cavity Passage into the upper airway/larynx, through vocal chords; if this happens (which may be suspected if the child has difficulty breathing or has a muffled voice), the NGT must be removed immediately.

### **In the event of complications:**

If the patient begins to vomit during the insertion of the tube, skip the initial supine decubitus position and go directly to the right or left decubitus lateral position.

**To verify that the tube is in the stomach**, auscultate for a gurgling sound over the epigastrium or left upper quadrant of the abdomen as air is injected through the NGT with a syringe. The child's voice/cry should be normal and strong.

### **Securing the NGT**

Secure the NGT by: Applying soft (e.g., paper) adhesive tape to the patient's ipsilateral cheek

Placing the NGT over the paper tape and fixing it with a piece of strong tape to the paper tape so that no contact is made with the skin

Fix the remaining NGT with tape to the shirt / pajamas in the anterior axillary line.

### **Gastric aspiration**

Immobilize the child.

After immobilizing, remove the tape that fixed the NGT in order to facilitate mobilization of the NGT.

With the NGT at the marked position (“neutral position”), aspirate with a syringe (20 mL syringe is preferable, as larger syringes may cause collapse of the NGT).

While withdrawing the NGT by 2 to 4 cm (“out position”), aspirate simultaneously to demonstrate aspiration of gastric contents.

Insert the tube 2 to 4 cm beyond the mark (“in position”) and aspirate, simultaneously until more gastric content is retrieved.

Place the gastric aspirate into the sterile wide mouth bottle

If less than a total of 3 mL of gastric juice are aspirated: Instill 20 mL of sterile water through the NGT

Wait at least one minute

Aim to recover at least half of the instilled volume (usually, at least 5 mL are retrievable),

Place the gastric aspirate in the same container.

Discard samples containing [undigested] food

After removal of the tube, unwrap child and return to parents.

### **Transport:**

Transport to the laboratory as soon as possible .In case of delay refrigerate the sample as soon as possible to prevent bacterial overgrowth

**Rejection Criteria:**Sample less than 5 ml

Sample containing undigested food

Sample without appropriate label.

## **6. 12. Genital tract female – cervical swab**

### **Container Specification/Materials required:**

Sterile swab and test tube

### **Method of collection :**

Using a speculum collect specimen from

Endocervical region / Posterior fornix.

**Transport:**

Transport to the laboratory as soon as possible.

**Rejection Criteria:**

Sample without appropriate label.

Requisition not made on HMIS

**6.13. Hair/Nail clippings . skin scrapings for mycology**

**Container Specification/Materials required:**

Clean Paper, blunt scalpel ,scissor/ nail clipper wiped with spirit

**Method of collection :**

Clean the skin with methylated spirit, and from the edge of the margin collect a scrapping .

Collect a nail clipping from affected area.

Pluck a hair from adffected area .

All these specimens can be placed in a clean paper,wrapped and sent to the laboratory.

**Transport:**

Transport to the laboratory as soon as possible.

**Rejection Criteria:**

Sample without appropriate label.

Requisition not made on HMIS

**6.14.Sputum: Container Specification/Materials required:**

Sterile wide mouth container

**Method of collection**

Spontaneous sample:

Should be an early morning sample.

Collected after rinsing the mouth with water.

Patient is instructed to take a deep breath and collect the sample from the chest.

Patient should collect the specimen either outside in the open air or away from other people and not in confined spaces such as toilets.

**Induced sputum**

These specimens resemble saliva but have to be processed as adequate specimens.

**Transport:** To the laboratory as soon as possible . If culture for tuberculosis is required send samples to the RNTCP centre in the OPD before 1 pm

In case of delay refrigerate the sample as soon as possible to prevent bacterial overgrowth.

**Special Instructions:**

If patient has difficulty expectorating then collect sample after nebulisation

Mention the test required eg routine culture or culture for mycobacteria.

Do not divide the sample send it in one container,but mention all the tests required

**Rejection criteria:**

Sputa from suspects should be rejected only if they are liquid and as clear as water, with No particles or streaks of mucous material. However, they should be accepted if the patient cannot produce a better specimen on a repeated attempt. Sputa from follow-up patients should be accepted and examined even if they look like saliva, since these patients often cannot produce mucoid specimens.

**6.15. Tracheal aspirate :**

**Container, Specification/Materials required:**

Do NOT send catheter tips. Ask Lab for special container (Suction trap)

**Method of collection :**

Insert suction trap between the suction tube and ventilator

**Transport:**

Send to the laboratory as early possible (within a few hours) .

**Rejection Criteria:**

Catheter tips.

Sample without appropriate label.

Requisition not made on HMIS/Form.

**6.16 .Throat Swab:****Container Specification/Materials required:**

2 Sterile swabs and a sterile tube

**Method of collection**

Ask patient to open mouth wide

With proper lighting and without touching the sides of the cheek collect 2 swabs from the infected area.

Both swabs can be transported in one sterile tube.

**If diphtheria** is suspected collect sample from the edge and under the pseudomembrane.

Collect atleast 3 swabs or make 2 smears and transport a separate swab in tube for culture.

**If swine flu** is suspected ask for Viral transport media (VTM) and sterile swabs. Collect nasal swab from both nostrils and one throat swab. Transport in same VTM.Fill the history sheet (NIV does not accept samples if the history is incomplete) All sample received before 10 am are sent to NIV and report is available on the same day. Samples which come later will be refrigerated and sent the following day

**Transport:**

Transport to the laboratory as soon as possible.

If delay is expected refrigerate sample

**Rejection criteria-** Dry swabs, Label not matching with that of online requisition or lab requisition form in case online requisition is not there.

**6.17. Urine:****Container Specification/Materials required:**

Sterile wide mouth container

**Method of collection**

Should be an early morning sample.

Resident should instruct the patient to wash genital area with soap and water following which the patient should collect a mid stream sample.Only 5ml is required . The outside part of container should not be soiled

**For AFB culture:** Contact lab before collecting sample for appointment.Collect fully voided mid stream sample on 3 consecutive days in sterile container

**DGI for leptospira:** 5 ml of fresh urine sample to be alkalanised with1ml  $\text{NaHCO}_3$  .If urine is not alkalanised leptospira die and cannot be seen.

**Transport:**

Transport to the laboratory as soon as possible.

If delay is expected refrigerate sample max delay 2hrs.

**Rejection criteria:** Containers that are non-sterile or cannot be tightly sealed or is leaking or the container is contaminated from outside.

**6.18. Urine from Catherised patient :**

**Container Specification/Materials required:**

Sterile test tube or wide mouth container

**Method of collection** Clamp the catheter drainage pipe, and allow urine to fill up (can take long). Sterilise the drainage arm of the foley's catheter with 70% alcohol, and with a sterile needle and syringe collect 5ml of urine. Transfer to a sterile tube or wide mouth container . Mention the sample is from a patient with a catheter

**Transport:**

Transport to the laboratory as soon as possible.

If delay is expected refrigerate sample for upto 2hrs.

**Rejection criteria:**

Containers that are non-sterile or cannot be tightly sealed or is leaking or the container is Contaminated from outside.

**6.19. For Catheter related blood stream Infection (CRBSI):****Container Specification/Materials required:**

Sterile Blade, Sterile test tube and blood culture bottle .

**Method of collection**

With a sterile blade cut the catheter tip and allow it to drop into a sterile test tube.

At the same time collect a peripheral blood culture after taking necessary precautions.

**Transport:**

Transport to the laboratory as soon as possible.

If delay is expected keep at room temperature

**Rejection criteria:**

Catheter tip without a blood culture sample.

**6.20. Genexpert for Tuberculosis :****Container Specification/Materials required:**

Sterile test tube. For tissue sterile tube with sterile Normal saline.

**Method of collection**

For M. tuberculosis: CSF /tissue/pus/sputum, BAL same samples except tissue for **Gene expert**

**Transport:**

Transport to the laboratory as soon as possible.

If delay is expected keep refrigerated.

**Rejection criteria:**

History form incomplete

**6.21 Bronchoalveolar Lavage****Container Specification/Materials required:**

Sterile wide mouth container

**Method of collection :**

Collect sample with necessary aseptic precautions

**Transport:**

Transport to the laboratory as soon as possible .

If culture for tuberculosis is required send samples to the RNTCP centre in the OPD before 1pm in case of delay Refrigerate the sample as soon as possible to prevent bacterial overgrowth.

**Rejection Criteria:**

Dried up sample

Sample without appropriate label.

Requisition not made on HMIS /Form

## 6.22 Histopathology specimen

### **Container Specification/Materials required:**

Wide mouth screw cap container - as per size of specimen with 10% formalin.

Liver biopsy for glycogen storage disease in 70% alcohol.

Bone marrow biopsy and liver biopsy in Bouin's fluid.

### **Transport:**

Transport to the laboratory as soon as possible .

### **Rejection Criteria:**

Specimen not in formalin

Sample without appropriate label.

Mismatch or wrong labelled sample

Incompletely filled requisition forms

Requisition not made on HMIS /Form

**7.0 List of Activities:** The list of activities or tests that are performed in microbiological laboratory is given in annexure # 1

**8.0 Patient identification:** It is important that the patient be specifically identified by allotting unique numbers to them. The unique number given by hospital to the patient attending the hospital is the called the MRD no.

### **9.0 Counselling the patient**

It is mandatory that each patient should be counselled properly before the collection of sample.

He/ She should be informed about the reason for collecting the, brief procedure of collection and need for the testing.

He/she should be provided with all requisite information pertaining to HIV like routes of transmission, preventive measures, etc.

### **10.0 Consent**

An appropriate written consent should be obtained from the patient whose HIV testing is to be done in a prescribed format after counseling the patient (Annexure# 2).

**11.0 . Test requisition:** The requisition for the test is sent through the HMIS system which has been installed in the hospital as in the laboratory. The following details are filled by the resident doctors/Physician

- a. Name of the patient
- b. MRD Number
- c. Age/Gender
- d. OPD/WARD NO
- e. Number of visit
- f. Date and Time of collection
- g. Type of specimen/ Site
- h. Brief History of Patient
- i. Examination Required
- j. Sender's and clinician's name
- k. The sample is received with the MRD no. written on it. On receipt of the sample, its verification is done regarding its online requisition on HMIS and then the sample is received in central receiving counter.

We do not receive any **requisition form until and unless there is HMIS soft ware break down**

### **12.0 Verbal orders and add-on-tests (additional examination)**

Telephonic or verbal orders are not entertained by the laboratory

### **13.0 Transportation and Storage-**

All specimens should be transported to the microbiology laboratory within 1 hour of collection.If after collection delay in transportation is unavoidable then these specimens can be refrigerated at 2-8<sup>0</sup>C for one

week. But specimens like CSF (where *Streptococcus pneumoniae* or *Haemophilus influenzae* is suspected) should never be refrigerated. Use gloves during transfer of samples. Ensure that the samples are transferred in upright position to avoid leakage and spillage. Ensure that the sample is handed over to the authorized person in the sample collection centre and should not be left unattended.

#### **14.0 Receiving of samples:**

Here in microbiology laboratory specimens are received in the central receiving counter where it is checked for rejection criteria and then received. On receiving, its entry is made in a register; the laboratory attendant should first sign in the register and then bring them to the respective laboratory on an ice pack as soon as possible in a leak proof closable plastic container with biohazard sign.

Specimens transported from or to off site clinic are received in triple packing system

Refer SOP on Laboratory Safety Manual #

#### **15.0 . Urgent samples**

In case where urgent report is required, mark “URGENT” on the transport sheet and inform the concerned laboratory about the urgency over telephone. The clinician should write a note on the transport sheet, if applicable.

The concerned laboratory after receiving the sample should confirm that the sample received is in good condition. The laboratory technician in the laboratory should cross check the sample, labels and transport sheet before processing the sample.

After checking, the sample should be processed immediately and the report should be informed to the laboratory In charge /supervisor

The report should be informed immediately to the Clinic technician/Clinician over the telephone and the printed report should be sent later.

A register should be maintained for all the reports that are conveyed telephonically to the clinics. The register should include the information as to the details of the participant, report details, time of reporting, concerned person in the clinic to whom report given, and the details and sign of the person giving the report from the laboratory.

**16.0 Samples Received outside routine working hours:** The routine working hour when all microbiological samples are received in the collection centre at the department is 10:00 am to 1:00 pm. From 1:00pm to 5:00 pm samples are received in individual sections of the department. Samples received before 10:00 am and after 5:00 pm are processed at the emergency laboratory located at the Central clinical laboratory (CCL) located at the first floor of the hospital building. The samples that are received at the emergency laboratory are gas gangrene smear or panophthalmitis samples for gram stain and hanging drop preparation for cholera. The samples are to be handed over to the authorized person only (on-call residents).

#### **17.0. TURN-AROUND-TIMES (TATs)**

Estimated turn-around-times for testing are given in **Annexure#1** On many occasions the results will be available earlier than the times stated and on occasions it could take longer, depending on the urgency and complexity of the work undertaken. If a result is required urgently, the requisition will be marked “URGENT” and same will be conveyed to laboratory.

#### **18.0 REPORTING RESULTS**

All the test results are recorded and verified in the register in standard format by laboratory supervisor/designee. These reports are dispatched on line on HMIS by the Laboratory supervisor.

The results of HIV testing are entered in a standard reporting format by the ICTC Laboratory Technician (**Annexure# 3**). The Laboratory Supervisor or SRL Incharge verifies the report with signature and stamp. The reports are released by the counsellor on the same day after 3:30 pm. Emergency testing and reporting (after and before duty hours) is not available at the ICTC center. Telephonic or verbal reporting of HIV test results are not entertained by the ICTC laboratory. But for rest other testing verbal or telephonically reporting of results is done. But in that case the name of the person to whom it is reported is written in the register.

## 19.0 REFERENCES-

Manual on Quality Standards for HIV Testing Laboratories March 2007

Laboratory Technicians; Revised National Tuberculosis Control Programme. Ministry of Health, India. March 2006.

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Koneman DW, Allen SH, Janda MW. Editor. Color atlas and text book of diagnostic microbiology, Koneman sixth edition. Lippincott publications. , Philadelphia, 1997;1: 222

### Annexure 1 List of tests offered at clinical service laboratory along with the Turn around Time (TAT)

Test code	Test name	Specimen	Container	Volume (ML)	Notes	TAT	Method
1.	HIV Rapid	Blood	Red top	3-5ml Adult 1-3ml Children	-	1 day	Rapid test- Tri spot, SD bioline, Comb-AIDs
2.	CD4/CD8	Blood	Violet	1-3 ml		1 day	FACS calibur
3.	Gram stain	Pus, Urine, Sputum, Genital & Oral swabs	Universal container and swabs in specific transport tubes	2-3 ml 2 swabs	-	Same day	Microscopy
4.	Gram stain for gas gangrene	Slide with the smear	NA	NA	-	1 hour	Microscopy
5.	ZN stain	Pulmonary and extrapulmonary specimens	Universal container	3-5 ml	-	1 day	Microscopy
6.	Flouochrome stain	Pulmonary and extrapulmonary specimens	Universal container	3-5 ml	-	1 day	Microscopy
7.	Modified ZN	Stool	Universal container	1-2 g	-	1 day	Microscopy
8.	Dark Ground Illumination	Urine, Skin discharge on the slide with cover slip in wet chamber	Urine- Sterile test tube	2-3 ml	Transport immediately	Same day	Microscopy
9.	Albert Stain/KLB report	Throat swabs	Sterile test tube	2 swabs	-	2-4 hrs	Microscopy

10.	KOH mount	Tissue sample	Sterile test tube/Bulb with sterile normal saline	NA	-	1 day	Microscopy
11.	Hanging drop preparation	Watery stool sample	Universal Container	2-5 ml	Transport immediately	2-4 hrs	Microscopy
<b>Test code</b>	<b>Test name</b>	<b>Specimen</b>	<b>Container</b>	<b>Volume (ML)</b>	<b>Notes</b>	<b>TAT</b>	<b>Method</b>
12.	India Ink preparations	CSF samples	Sterile test tube	2-5 ml	-	1 day	Microscopy
13.	Bacterial culture & Sensitivity	Pus, Urine, Sputum, Genital Oral swabs, throat swabs body fluids, CSF, blood	Universal container and swabs in specific sterile test tubes	2-5 ml Swabs: 2 no.	-	1 day for negative report Blood culture- 5-10 days For other positive culture 3-4 days	Culture
14.	Fungal culture	Pus, Urine, Sputum, Genital/ Oral / throat swabs body fluids, CSF, blood	Sterile containers	2-5 ml Swabs- 2 no.	-	2-7 days for yeast 2-4 weeks for molds	Culture on SDA
15.	Mycobacterial culture	Pulmonary and extrapulmonary specimens	Sterile Sputum container	2-5 ml	-	8 weeks for negative report 3-4 weeks for positive report	Conventional Culture method on LJ
16.	Antimycobacteria l sensitivity testing	Culture isolate	NA	NA	-	42 days after the growth	Proportion method on LJ
17.	MGIT culture ( liquid)	Pulmonary and extrapulmonary specimens	Sterile container	2-5 ml	-	42 days for negative report 5-14 days positive report	MGIT
18.	DST by MGIT	Culture isolate	NA	NA	-	4-14 days	MGIT
19.	Bactec for bacterial, fungal, TB	Blood	Bactec Containers	8-10ml Pediatric-1-3 ml	-	Bacterial Positive- Within 24 hrs after it beeps positive Negative-5 days TB/Fungal-Positive -5-14 days Negative-42 days	Bactec

20.	Serological assays- VDRL	Serum/Plasma	Bulbs or red Vacutainer	2 ml	-	1 day	Slide flocculation test
21.	Serological Assays- ASO,CRP,RA, Malarial antigen,	Serum/ Plasma	Bulbs or red Vacutainer	2 ml	-	1 day	Rapid test
22.	Widal	Serum/ Plasma	Red Top	2 ml	-	2 day	Tube agglutination test
<b>Test code</b>	<b>Test name</b>	<b>Specimen</b>	<b>Container</b>	<b>Volume (ML)</b>	<b>Notes</b>	<b>TAT</b>	<b>Method</b>
23.	Serological Assays- a.HBsAg b. Dengue IgM c. Chikungunya IgM	Serum/ Plasma	Bulbs or red Vacutainer	2 ml	-	a) 4 days b) 1wk c) 1wk	ELISA
24.	Cryptococcal Antigen Detection	CSF, BAL, Serum	Sterile container	2-5 ml	-	2 hrs	CALAS Latex agglutination test
25.	TB PCR	Pulmonary and extrapulmonary specimens	Sterile container	1- 5ml	-	2 days	PCR- MPB 64 antigen
26.	GeneXpert	Pulmonary and extrapulmonary specimens	Sterile container	0.5-5ml	-	4 hrs	Real time PCR
27.	Histopathology Examination ( H & E stain )	Surgically removed specimen and biopsy	Appropriate Size wide mouth screw cap container with 10% formalin		NA	7 days	Light microscopy on H & E stain
28.	Blood Urea	Serum	Red top	3ml		6hrs	GLDH method
29.	Serum Creatinine	Serum	Red top	3 ml		6hrs	Modified Jafee's Method
30.	Blood Sugar	Serum/ Plasma	Red top/Grey top	3 ml	Fasting :8hr fasting PP: 2hrs after meal Random	6hrs	Hexokinase method

<b>31.</b>	Total T3 Total T4 TSH	Serum	Red top	3ml	Fasting for 8hrs	48hrs	Chemilumines- cence method