SPECIMEN COLLECTION MANUAL

MAULANA AZAD MEDICAL COLLEGE

Department of Microbiology

Edited by
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Message from Dean

It gives me great pleasure to introduce this Microbiology Sample Collection Manual—an essential resource developed to uphold the highest standards in diagnostic healthcare.

In an era where timely and precise microbial identification is critical to both individual patient care and public health, particularly amidst the growing threat of antimicrobial resistance (AMR), the significance of proper specimen collection cannot be overstated. This manual serves not only as a technical reference but also as a reflection of our institution's commitment to clinical excellence, academic rigor, and continuous improvement in healthcare delivery.

The effort of our Microbiology department in creating this guide underscores our shared mission: to advance patient outcomes through evidence-based practices and standardized protocols. I encourage all members of our healthcare community—students, clinicians, nurses, and laboratory personnel—to utilize this manual as a reliable tool for both learning and practice.

As Dean, I take pride in the collective effort behind this work and trust it will contribute meaningfully to the education of our future professionals and the care of our patients.

With best wishes

Dr Poonam Narang

Dean

Message from Medical Director

Accurate and timely diagnosis is the cornerstone of effective patient care, and the role of microbiological testing in this process is indispensable. However, the quality of microbiological results is only as good as the quality of the specimen collected. Proper specimen collection, handling, and transportation are critical steps that directly impact the reliability of laboratory testing, the interpretation of results, and ultimately, patient outcomes.

This manual has been developed to serve as a comprehensive guide for all healthcare professionals involved in the collection of microbiological specimens.

I congratulate the department of Microbiology for creating a document that will be immensely useful for all. I encourage all users to familiarize themselves with the contents of this manual, refer to it regularly, and seek clarification whenever needed.

Dr B L Chaudhary
Medical Director

Forward

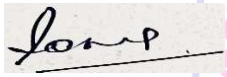
Accurate microbiological diagnosis begins long before a sample reaches the laboratory—it begins with correct collection practices at the bedside, in the clinic, or in the field. Recognizing the importance of this critical first step, we have developed this Microbiology Sample Collection Manual to serve as a clear, concise, and practical guide for all healthcare professionals involved in specimen collection.

This manual outlines the best practices for collecting a wide range of microbiological specimens, with a strong emphasis on proper technique, asepsis, transport conditions, and documentation.

I urge all users to treat this manual as a living document—one that reflects current evidence-based practices and that will evolve with scientific advancements and clinical needs.

I thank everyone who contributed to this manual and encourage its widespread use as part of our ongoing commitment to patient safety, clinical excellence, and education.

Warm regards



Dr Sonal Saxena

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1.0. Instructions to clinicians and selection of tests

- Refer to the list of microbiology laboratory services provided to find whether a test intended is available or not before collecting the sample.

1.1 Timing for collection

a. **For inpatients**: Specimen collection centre, Ground floor, Room no. 30, Department of Microbiology, Pathology block from Monday to Friday 9 a.m. – 3 p.m. and Saturday 9-11 a.m.

b. **For OPD**: Specimen collection centre, First floor, OPD block, Monday to Friday 9 a.m.-12 noon and Saturday 9-11 a.m.

c. **Hepatitis makers** in Collection Centre in ANC from 9-11 a.m.

d. **For emergency specimens after 3 p.m.**, all 7 days: Emergency Laboratory of Microbiology, 1st floor, LNH

e. **ICTC**: HIV Monday to Saturday: 9-11:30 a.m.

f. **Water bacteriology**: Room 124A, Enterobacteriaceae Lab, Department of Microbiology, MAMC. Monday to Thursday: 9 a.m. – 12 p.m. (Only with prior intimation to Dr Prabhav Aggarwal, Faculty Lab In-charge)

Table 1: LIST OF MEDICAL SERVICES PROVIDED BY MICROBIOLOGY DEPARTMENT

S. No	Name of Test	Specimens	Container
1.	Gram stain	Pus, CSF, urine, sterile body fluids, sputum, BAL, endotracheal aspirate, tissue biopsy	Slide/ Two swabs/ samples in a sterile container
2.	Special stain (Albert's, Giemsa, Modified ZN stain)	Throat swab (albert's stain), stool (modified ZN stain)	
3	Acid Fast Stain/ Ziehl-Neelsen staining	All specimens suspecting M. tuberculosis/ NTM	Sterile leak proof container
4	KOH Preparation	Cutaneous samples (Hair, skin & nail), Specimens suspecting fungal etiology	
5	India ink preparation for Cryptococcus species	Cerebrospinal fluid	
6	Aerobic Culture and antimicrobial susceptibility	Urine, Pus, CSF, sterile body fluids, sputum, BAL, endotracheal aspirate, Tissue biopsy, stool, ocular specimens, ear discharge	
7	Culture for Mycobacterium TB – MGIT	All specimens suspecting M. tuberculosis/ NTM	
8	Anaerobic culture and antimicrobial susceptibility	Pus, CSF, sterile body fluids, endotracheal aspirate, tissue biopsy, ocular specimens	

9	Bacterial/fungal Culture and antimicrobial susceptibility for Blood	Blood	Blood culture bottle
10	Aerobic Culture and susceptibility for swab	Wound swab, throat swab, nasopharyngeal swab, cervical swab	Sterile swab
11	Fungal Culture and antifungal susceptibility for yeasts	Cutaneous samples (Hair, skin & nail), Specimens suspecting fungal etiology	Sterile leak proof container/ petri dish/ filter paper
12	Bacteriological assay of Water	Water from various sources for testing	Water sample in sterile bottles – 100ml, 50 ml
Serology/Immunology			
13	Anti-CCP antibodies	Blood	Yellow/ Red cap Vacutainers
14	Serum Procalcitonin		
15	Widal test		
16	Rapid test for typhoid fever – IgM		
17	Anti-Streptolysin O test (ASO)		
18	C Reactive Protein (CRP)		
19	Rheumatoid Factor (RA test)		
20	HBs Antigen detection rapid test		
21	Anti-HBs antibodies		
22	IgM antibody detection for HAV (ELISA)		
23	IgM antibody detection for HEV (ELISA)		
24	Anti-HCV antibodies rapid test		
25	IgM and IgG ELISA for TORCH		
26	IgM ELISA for Chikungunya		
27	NS1 antigen ELISA - for Dengue		
28	IgM ELISA - for Dengue		
29	Rapid test for Leptospira IgM antibody		
30	Serum Galactomannan rapid test		
31	Candida IgM antibody rapid test		
32	EBV- VCA IgM, IgG & IgG avidity EA & NA IgG		
33	HSV-1 & HSV-2 IgM		
34	Toxoplasma IgG and IgM ELISA		
35	Entamoeba histolytica IgG ELISA		

36	Hydatid serology	Blood	Yellow/ Red cap Vacutainers
37	ANA IFA & LIA		
38	APLA, ANCA, anti-CCP, anti-dsDNA (ELISA)		
39	Immunological tests for autoimmune hepatitis, myositis & encephalitis		
40	C. difficile GDH and toxin A and B detection by ICT	Stool	Sterile leak proof container
41	Entamoeba histolytica, Giardia lamblia & Cryptosporidium antigen by ICT		
42	Occult blood by Guaiac hemospot method		
43	Helicobacter pylori antigen by ICT		
44	Rotavirus/ Adenovirus antigen by ICT		
46	Histoplasma antigen test	Urine	
47	Malaria/ Filaria ICT	Blood	EDTA Vacutainers
48	Peripheral smear: Malaria/filaria		Peripheral smear/ EDTA Vacutainers
Molecular Tests			
49	RT PCR – SARS-CoV-2/ Respiratory panel	NPA/ Throat swab/ Nasal swab	Swab in VTM
50	Dengue virus serotypes	Blood	EDTA Vacutainers
51	HCV RNA Qualitative & genotyping		
52	HBV RNA Qualitative & genotyping		
53	Scrub typhus		
54	Acute undifferentiated fever panel		
55	Human EBV detection (quantitative)		
56	Zika virus		
57	Viral & Bacterial meningitis/ encephalitis panel	Blood/ CSF	
58	JE/ Hantavirus/ Measles	Blood/ CSF	
59	Human CMV detection (quantitative)	Urine & blood	
60	Mpox PCR	Lesion swab/ Blood/ Urine	EDTA Vacutainers/ Sterile leak proof container
61	Rabies PCR	Blood/ Saliva/ Urine/ Tissue	
62	HPV genotyping	Pap/ Liquid based cytology	Sterile leak proof container
63	Gastrointestinal panel	Stool	

2.0. Patient identification

- Correct identification is essential for patient safety. Each patient must be identified positively, using active communication techniques by means of **two patient identifiers** prior to collection of samples.
    - Patient's name
    - Hospital UHID/ CR. No.

In an in-patient setting, the patient's room number or physical location should NOT be used as an identifier.

- The identifying label must be attached to the sample container(s) at the time of collection. The containers used for laboratory samples should be labelled with the identifiers in the presence of the patient.
- Clear explanation to the patients about the laboratory tests and how they will be collected should be communicated to patients.

3.0. Labelling

The test requisition form (TRF) should accompany the specimen and it should be placed in a separate place outside the transport box to prevent smudging on account of spillage.
Mandatory information needed on all patient requisitions on TRF and sample container.

Test Request Form The test request must be made in Microbiology – Test Request Form (TRF) and the following details must be filled in:	**Labelling of Specimen Containers** Label all sample containers prior to collection at the patient's side. The following information is mandatory:
• **Patient's name** • **Patient's identification Number** • **Date and Time of Sample Collection** • **Nature of Sample**: Source of sample • **Name and Details of Ordering Doctor:** Details of the requesting doctor (i.e., name, designation and department and unit) should be included in the requesting form. • **Clinical History, Age and Gender:** Include the clinical diagnosis, suspected disease/organism, brief clinical history, name, date and duration of treatment given, previous test results with dates and previous laboratory numbers, patient's immune status (e.g., any underlying diseases, cancer chemotherapy, immunosuppressive treatment), and any other relevant patient or clinical data.	• Patient Name • Patient ID • Department + Unit + Location • Date and time of Sample collection • Sample ID given by laboratory (as soon as it is generated) • Stick the label lengthwise. • Unlabeled samples will be rejected.

4.0. Types of specimens

- Blood, serum, plasma: Venipuncture or Central line catheter
- CSF: Lumbar puncture
- Pus: Aspirated, swab
- Respiratory specimen: Tracheal aspirate, Sputum, BAL, Nasopharyngeal aspirate, Nasopharyngeal swab, Throat swab,
- Sterile body fluids: Pleural fluid, Peritoneal fluid, Synovial fluid, Pericardial fluid
- Cutaneous specimens: Hair, Nail, Skin scraping.
- Urine: Mid-stream sample/ Catheter sample/ Suprapubic aspirate/ First-void urine
- Ocular: Corneal scraping, ocular swabs
- Cervical swab, high vaginal swab
- Water bacteriology
- Toxin detection of Dialysate
- Stool
- Tissue

5.0. General instructions for specimen collection for microbiology tests

- High-quality and appropriately collected samples are crucial for reliable and prompt laboratory results.
- All samples should be regarded as potentially infectious and the standard precaution guidelines should be adhered by all healthcare workers during sample collection and handling.
- Collect samples where possible prior to starting antibiotics.
- Samples must be collected into appropriate containers approved by the microbiology department.
- Collect sufficient amounts of the specimens to enable the test(s) to be carried out, especially when multiple tests are ordered. In the case the amount of sample is insufficient please state which tests should be done in order of priority.
- Please check the containers again after sample collection for any leakage and tighten the lids of containers properly to prevent leakage of samples during handling and transportation. A leaked sample container can pose infection hazards to the transportation and laboratory staff.
- Any spillage of samples should be treated as per spill management policy of the hospital.
- Ensure that the sample has been labelled correctly and the appropriate test requisition form (TRF) has been filled. The TRF should not be packed with sample
- After collection discard all PPE and other contaminated materials as per hospital's biomedical waste management policy.
- Specimens to be transported to the laboratory as soon as possible.
- Transport specimens /swabs in a suitable transport media wherever indicated.

6.0. Procedure for specimen collection for various microbiological tests

6.1. Blood for culture

6.1.1. Timing and Volume of collection

- Collect strictly before starting the antibiotics.
- If a patient is already on antibiotics, then collect just before the next dose of antibiotic, as the antimicrobial agents are at their lowest concentration.
- If a patient has fever, then collect before or during the fever spike (based on the temp chart), since the number of bacteria is higher at high temperatures.
- Collect blood during the early stages of disease since the number of bacteria in blood is higher in the acute and early stages of disease.

6.1.1.a Paired aerobic/anaerobic blood cultures

- A paired culture sample refers to two blood culture specimens that are **collected from separate venipuncture sites, ideally at the same time or within a short interval** (usually within 15–30 minutes), to improve the diagnostic yield and help differentiate between true bloodstream infection and contamination.
- One sample is typically drawn peripherally, while the other may be taken from a central line or another peripheral site.

6.1.1.b. Recommended total volume and numbers of Conventional blood cultures

6.1.1.b.1 Adults

- 30 to 40 ml of blood to be divided between **two blood cultures**.
- At least 20 to 30 ml of total blood in two draws is the minimal requirement
- Draw out 5ml of blood for one blood culture bottle containing 50ml of BHI broth.

6.1.1.b.2. Pediatrics

- Small children usually have higher number of bacteria in their blood as compared to adults and hence less quantity of blood needs to be collected from them.
- Roughly 1 ml of blood per year of age, divided between two blood cultures having 20 ml of liquid media (Table 2).

Table 2: Volume of blood to be collected from children

Age	Volume in 2 bottles (ml)
neonates (<4 kg)	0.5-1 ml
1-2 years	1 ml
<2 years	2
2-5 years	8
6-10 years	12
>10 years	20

6.1.1.c. Automated (BacT/Alert) blood culture bottle options and recommended volumes as per bottle

- Aerobic Standard: 8 - 10 ml blood
- Anaerobic Standard: 5 - 7 ml blood
- Peds Plus: 1 - 3 ml blood. This bottle serves as the aerobic bottle of the set for pediatric patients due to the smaller volume requirements. This bottle may also be used in place of the aerobic bottle if the patient is known to currently be on antibiotic therapy, as the peds plus bottle contains an antibiotic-binding resin.

6.1.1.d. Special cases

- *Acute sepsis/osteomyelitis/meningitis/pneumonia/pyelonephritis*: Collect two blood cultures of maximum volume consecutively from different anatomic sites before starting antibiotics. Initially obtain three blood culture sets within a 30-minute period before administration of empiric antimicrobial agents from patients presenting with possible infective endocarditis. If those sets are negative at 24 hours, obtain two more sets of cultures, for a total of five sets overall.
- *Fever of unknown origin/subacute bacterial endocarditis/suspected bacteremia or fungemia*: Collect a maximum of three blood cultures with maximum volume.
- *Sample from indwelling catheter*: If poor access requires that blood for culture be drawn through a port in an indwelling catheter, disinfect the access port with a 2% chlorhexidine in 70% isopropyl alcohol impregnated swab for 30 seconds and a second culture must be from a peripheral site, because cultures drawn through catheters can indicate catheter colonization but may not be indicative of sepsis.

6.1.1.e. Precautions

- Blood samples should be obtained from a catheter only when peripheral venipuncture is not feasible and no alternative access sites are available.
- Do not draw blood from a vein into which an intravenous solution is running.
- It is not necessary to discard the initial volume of blood or flush the line with saline to eliminate residual heparin or other anticoagulants.
- Blood culture volumes should be limited to less than 1% of total blood volume (usually about 70 ml/kg). e.g., total sample limit would be 7 ml for a 10 kg patient and 28 ml for a 40 kg patient.

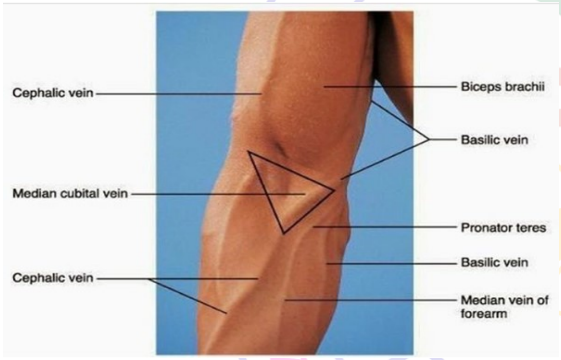
6.1.1.f. Collection

Skin preparation

- Clean hands using correct hand hygiene technique (use of the WHO ‘6 steps of hand washing’ at the ‘5 moments of hand hygiene’ is recommended).
- Select the site for venipuncture. Select a different venipuncture site for each blood culture.
- Clean any visibly soiled skin on the patient with soap and water.
- Apply a tourniquet 3-4 inches above the intended site of venipuncture, palpate to identify vein.

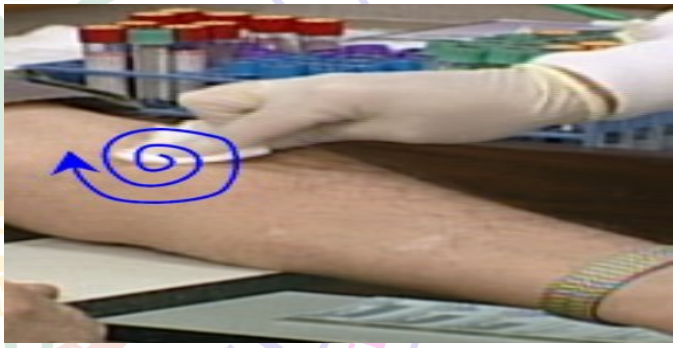
- Wear examination gloves
- Cleanse the selected venipuncture site.
- Vigorously cleanse with 70% isopropyl or ethyl alcohol to remove surface dirt and oils. Scrub the venipuncture site gently but firmly with the cotton beginning in the center and continuing in an outward direction circularly for an area of 4 to 5 inches in diameter and allow to dry.
- Apply a 1-2% povidone-iodine solution or 2% chlorhexidine gluconate (CHG) over the same area, in a similar manner as given earlier.
- Allow the povidone iodine/ CHG to dry (30 seconds)
- Cleanse the site a second time with an 70% isopropyl or ethyl alcohol to remove the iodine by wiping down the center of the prep area, then down each side. This step is helpful in the event the site must be palpated during the phlebotomy procedure.
- Do NOT touch the site after cleaning. Instruct patient to clench and unclench the fist.

Fig 1: Selecting the site for blood collection



(Source: https://in.pinterest.com/pin/328199891592997344/)

Figure 2: Clean the site of collection from inside-out.



(Source: https://www.phlebotomy.com/phlebotomyblog/standards- update-circular-cleansing.html)

Note: Skin preparation with either alcohol, alcoholic chlorhexidine (2%), or tincture of iodine (1%) leads to lower blood culture contamination rates than does the use of povidone-iodine. For pediatric patients, omit the iodine step and clean two additional times with separate preparation pads saturated with 70% isopropyl alcohol or ethyl alcohol

Collection of sample and inoculation of bottle

- Prepare the septum of the blood culture bottle and the rubber stoppers on bottles or tubes. Vigorously wipe septa with 70% alcohol and allow to dry completely, usually for 30 to 60 s. **This procedure should be completed before initiating any blood sample collection.**
- Collect sample and release tourniquet.
- Cover the puncture site with appropriate dressing.
- If blood is being collected for other tests, inoculate the blood culture bottles first.
- Inoculate first the aerobic bottle and then the anaerobic bottle with no more than the recommended amount of blood.
- Do not change the needle between sample collection and inoculation. Thoroughly mix bottles to avoid clotting.

- Inoculate blood into culture bottles; do not change the needle between sample collection and inoculation.
- Discard needle and syringe in a sharp container.
- Clean hands again using correct hand hygiene technique.

6.1.2. Blood for tests that require serum/ plasma

6.1.2.a. Collection

- Apply the tourniquet on forearm, select an appropriate vein (e.g. median-cubital).
- Use 70% ethanol /isopropyl alcohol / spirit swabs for cleaning the site in a circular pattern (inside to outside such that swab does not touch the same surface again).
- Puncture the selected vein, collect 3-5 ml of blood in a disposable syringe/ vacutainer.
- Collect the blood in plain vials (RED or YELLOW cap Vacutainer) for serum.
- After collection, slowly mix the blood by turning the tube in up-and-down fashion 1-2 times and keep it for about half an hour in test tube rack.
- Discard the syringe and needle as per BMW protocol.

6.2. Urine

6.2.1. Types of Specimens

a. Mid-stream clean catch urine specimen
b. Urine specimen from catheterized patient
c. Supra pubic aspiration
d. Per cutaneous nephrostomy aspirate

6.2.2. Specimen collection

a. Mid-stream clean catch urine specimen (most common sample)

- Urine sample is collected by the patient themselves so appropriate correct instructions must be given to patient for proper collection.
- Explain the concept of midstream to patient. Instruct the patient to collect voided urine directly into a disposable leakproof sterile container, instructing the patient to not halt and restart the urinary stream for a "midstream" collection but preferably move the container into the path of the already voiding urine.
- **Females :** Instruct the patient to wash the vulva thoroughly keeping labia apart from front to back with soap and water. Collection of midstream urine specimens should be avoided during menses.
- **Males:** Instruct to retract the foreskin, and wash the glans penis thoroughly with soap and water paying special attention to the urethral meatus if circumscribed, else the area can be washed prior to collection.
- **Never collect urine from a bedpan or urinal.**

b. Urine specimen from catheterized patient

- Clamp the catheter tubing
- Disinfect a portion of the catheter tubing using alcohol
- Puncture the tubing directly with a sterile syringe and needle

- Aspirate the urine and transfer to a sterile, wide mouth, screw capped universal container.
- Urine sample may also be obtained from the collection port in case catheters have a port.
- DO NOT collect from urobag

c. Supra pubic aspiration

- This method is the preferred method for infants, for patients for whom the interpretation of results of voided urine is difficult, and when anaerobic bacteria are suspected as the cause of infection.
- Urine collection by suprapubic needle aspiration directly into the bladder is performed by a physician or trained healthcare worker.
- Bladder should be full and palpable before aspiration.
- Shave and disinfect the skin over the bladder.
- Make a small lance wound through the epidermis above the symphysis pubis.
- Aspirate using a needle and syringe.
- Submit in syringe or sterile screw capped cup.
- Mention "suprapubic aspirate" clearly on the TRF.

6.2.3. Timing of specimen collection

- Obtain early-morning specimens whenever possible.
- Allowing urine to remain in the bladder overnight or for at least 4 h will decrease the number of false-negative results.
- Do not force fluids in order to have the patient void urine.
  Excessive fluid intake will dilute the urine and may decrease the colony count to 10^5CFU/ ml.

6.3. Cerebrospinal fluid

6.3.1. Specimen collection

Skin Preparation:

- Vigorously cleanse with 70% isopropyl or ethyl alcohol to remove surface dirt and oils. Scrub the site gently but firmly with the cotton beginning in the center and continuing in an outward direction circularly for an area of 4 to 5 inches in diameter and allow to dry.
- Apply a 1-2% povidone-iodine solution or 2% chlorhexidine gluconate (CHG) over the same area, in a similar manner as given earlier.
- Allow the povidone iodine/ CHG to dry (30 seconds)
- Cleanse the site a second time with an 70% isopropyl or ethyl alcohol to remove the iodine by wiping down the center of the prep area, then down each side. This step is helpful in the event the site must be palpated during the phlebotomy procedure.

Sample collection: Lumbar puncture

- Insert a needle with stylet at the L3-L4, L4-L5, or L5-S1 interspace depending on the age of the patient. When the subarachnoid space is reached, remove the stylet, spinal fluid will appear in the needle hub.
- Slowly drain the CSF in sterile universal leak proof container and transport the sample at room temperature.

6.4. Stool/ Rectal swab for culture

6.4.1. Specimen collection

i) Stool

- Provide the patient with a sterile wide mouth, screw cap, leak proof container.
- Instruct the patient to collect the freshly passed stool specimen directly into a wide mouth, screw cap, sterile leak proof container.
- It should not be contaminated with urine.
- Do not collect the specimen from bed pan.

ii) Rectal swab

- Alternatively, rectal swabs can be taken via a proctoscope.
- Moisten a cotton-tipped swab with sterile water.
- Insert the swab 2.5 cm beyond the rectal sphincter, rotate, and withdraw.
- Place the swab in an empty sterile tube with a cotton plug or screw-cap, if it is to be processed within 1–2 hours.
- If the swab must be kept for longer than 2 hours, place it in the appropriate transport medium such as Cary Blair medium.
- Rectal swabs are not recommended for viruses.

6.4.2. Stool for antigen detection

- Fresh stool sample must be transported laboratory immediately or refrigerated if there is delay.
- Fecal swabs are not acceptable.

6.5. Syndrome wise specimen collection

6.5.1.a. Upper respiratory tract infections

Syndrome	Most common organism suspected*	Specimen which can be sent
Pharyngitis	*Streptococcus pyogenes, Corynebacterium diphtheriae,*	Throat swab
Tonsilitis (Quinsy)	*Streptococcus anginosus, Streptococcus pyogenes, Fusobacterium necrophorum*	Pus aspirate from peritonsillar abscess
Epiglottitis	*Hemophilus influenzae type b, Streptococcus pyogenes, Candida sp.*	Blood culture
Laryngitis	*Corynebacterium diphtheriae, Streptococcus pyogenes, Streptococcus pneumoniae, H. influenzae*	Throat swab

* *Please note that the syndrome mentioned may be caused by other pathogens including viruses and fungi. Please refer to respective laboratory standard operating procedure to find the details of type of specimen and special conditions for specimen required, if any.*

6.5.1.b Specimen collection of upper respiratory tract infections:

Specimen collection	If Delay is anticipated
Throat swab: Use p**aired** sterile dacron or rayon swabs with plastic shafts. The inflamed areas of the throat, tonsils and then the pharynx are swabbed, taking care not to touch the lateral walls of the buccal cavity or the tongue to minimize contamination with commensal bacteria.	More than one hour use transport media
Peritonsillar abscess: Aspirate infected material with needle and syringe in a sterile leakproof container	

6.5.2.a. Lower respiratory tract infections

Syndrome	Organism suspected	Specimens which can be sent
Community acquired pneumonia	*Streptococcus pneumoniae, Hemophilus influenzae, Staphylococcus aureus*, *Legionella pneumoniae* rarely gram-negative bacilli like *Klebsiella pneumoniae* Most common causes are viral	1. Sputum (poor diagnostic value) 2. Bronchioalveolar lavage 3. Protected catheter specimens 4. Bronchial aspirate 5. Bronchial washings 6. Non-directed bronchoalveolar lavage (mini-BAL)
Hospital acquired pneumonia	Multidrug resistant gram-negative organisms are common such as *Pseudomonas aeruginosa, Acinetobacter baumannii*, Enterobacterales, MRSA, *Legionella sp.*	1. Bronchioalveolar lavage 2. Protected catheter specimens 3. Bronchial aspirate 4. Bronchial washings 5. Non-directed bronchoalveolar lavage (mini-BAL) 6. Tracheal aspirate
Lung abscess	*Staphylococcus aureus, Klebsiella pneumoniae, Nocardia sp., Burkholderia pseudomallei*	Bronchial aspirate Transthoracic aspirate

6.5.2.b Specimen collection for Lower Respiratory tract infections:

Specimen collection (Sterile leakproof universal container)	If Delay is anticipated
Expectorated Sputum: Food should not have been ingested for 1 to 2 hours before expectoration. Mouth should be rinsed with saline or water just before expectoration. Patient should be instructed to provide deep-coughed specimen.	Store at 2 to 8°C until cultures can be submitted or processed. Delay in processing of more than 1-2 hours may result in loss of recovery of fastidious bacteria
Endotracheal aspirate: Discard first aspirate. Collect the second aspirate after tracheal instillation of 5 ml saline in a mucus collection tube.	
Bronchoscopy samples (Bronchoalveolar lavage, Bronchial aspirate bronchial washings, transbronchial biopsy): To avoid excess blood in the recovery fluid, obtain bronchial wash and BAL specimens before brushings or biopsy specimens Bronchial brushing sample should be transported in 1ml of saline Lung biopsy: 1-3 cm^2 piece of tissue should be submitted in sterile container without formalin	

6.5.3.a. Ear infections

Syndrome	Organism suspected	Specimens which can be sent
Otitis Externa	*Pseudomonas aeruginosa, Alcaligenes faecalis, Staphylococcus aureus*	Pus swab
Otitis Interna	*Staphylococcus aureus, Pseudomonas aeruginosa*	Aspirate from middle ear

6.5.3.b Specimen collection for ear infections:

Specimen collection (Sterile leakproof universal container)	If Delay is anticipated
Malignant otitis externa: Insert sterile swab into ear canal until resistance is met and rotate swab and allow fluid to collect on swab.	Transport medium should be used
Otitis Interna: Clean the external ear canal. Using syringe aspirate the fluid from the ear drum. If eardrum is ruptured, collect the exudate by inserting a sterile swab through an auditory speculum	

6.5.4.a. Skin and soft tissue infections

Syndrome	Organism suspected	Specimens which can be sent
Folliculitis, Erysipelas, Ecthyma gangrenosum, Impetigo, Cellulitis	*Staphylococcus aureus*, Group A *Streptococcus, Pseudomonas aeruginosa, Stenotrophomonas maltophilia* (Ecthyma gangrenosum)	Pus swab Biopsy
Gangrene	*Staphylococcus aureus, Enterobacterales, Streptococcus sp., Pseudomonas aeruginosa*	Tissue

6.5.4.b Specimen collection for skin and soft tissue infections:

Specimen collection (Sterile leakproof universal container)	If Delay is anticipated
Pus Swab: Clean the superficial area with sterile saline, remove all superficial exudate. Remove overlaying debris with scalpel and swab or sponges. Collect biopsy or curette sample from the base or the advanced margins of the lesions.	More than one hour use transport media.
Tissue and Biopsy: Collect sufficient tissue, avoiding necrotic areas. Collect 3 to 4 mm biopsy samples.	

6.5.5.a. Sterile body fluid infections

Syndrome	Organism suspected	Specimens that can be sent
Pericarditis and peritonitis	Wide range of bacteria suspected in case of purulent pericarditis	Pericardial fluid, Ascitic fluid
Pleurisy	*Streptococcus pneumoniae, Cryptococcus neoformans*, NTM, MTB	Pleural Fluid Empyema
Septic arthritis	*Staphylococcus aureus, Streptococcus sp., Enterobacterales,* MTB, *Streptococcus pneumoniae, Kingella kingae*	Synovial fluid
Amnionitis	Polymicrobial	Amniotic fluid

6.5.5.b. Specimen Collection for sterile body fluids:

Specimen collection (Sterile leakproof universal container)	If Delay is anticipated
• Aspirate the fluid aseptically with syringe and needle • Use safety devices to protect from needle exposure • Collect 1-10ml of fluid in sterile A sterile vacutainer without preservatives can also be used. • Avoid using anticoagulants as they may inhibit the growth of the microorganism.	• Transport as soon as possible • Do NOT refrigerate • If delay is anticipated it is recommended to collect the sample in blood culture bottle and use about 0.5ml of sample to prepare a slide there only and then send it to the laboratory for culture and staining

6.5.6.a. Pus and exudates

Syndrome	Organism suspected	Specimens which can be sent
Brain abscess	Anaerobes are most common *Streptococcus pneumoniae, Enterobacterales, β-hemolytic streptococcus sp, Staphylococcus aureus*	Aspirate
Breast abscess	*Staphylococcus aureus, Pseudomonas aeruginosa, Proteus sp.*	Aspirate, Biopsy, Tissue
Dental abscess	Anaerobes are most common, α-hemolytic *Streptococcus sp.*	Aspirate
Liver abscess	*Enterobacteriaceae, Enterococcus sp., Pseudomonas aeruginosa, Burkholderia pseudomallei*	Aspirate, Biopsy
Lung abscess	*Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella sp.*	Aspirate, Biopsy
Pancreatic abscess	*Escherichia coli, Enterococcus sp., Coagulase negative Staphylococcus sp., Candida sp.*	Aspirate
Perirectal abscess	*Enterobacteriaceae, Streptococcus sp., Staphylococcus aureus*	Aspirate, Biopsy
Pilonidal abscess	*Enterobacteriaceae*	Biopsy, Aspirate
Prostatic abscess	*Escherichia coli, Enterococcus sp., Neisseria gonorrhea*	Aspirate
Psoas abscess	*Enterobacteriaceae, Streptococcus sp., Staphylococcus aureus*	Aspirate, Biopsy
Renal abscess	*Staphylococcus aureus*	Aspirate, Biopsy

6.5.6.b Specimen collection for pus and exudates

Specimen collection (Sterile leakproof universal container)	If Delay is anticipated
Pus Aspirate: The deepest portion of the lesion or exudate with the syringe and needle.	More than one hour use transport media
Tissue and Biopsy: Collect sufficient tissue, avoiding necrotic areas. Collect 3 to 4mm biopsy samples.	

6.5.7.a. Ocular infections

Syndrome	Organism suspected	Specimens which can be sent
Conjunctivitis	*Staphylococcus aureus, Streptococcus pneumoniae, Hemophilus influenzae, Moraxella catarrhalis, Enterobacterales, Pseudomonas aeruginosa, Neisseria gonorrhoeae, Chlamydia trachomatis*	Conjunctival swab Conjunctival scrapping
Keratitis	*Staphylococcus aureus, Streptococcus pneumoniae, Hemophilus influenzae, Moraxella catarrhalis, Enterobacterales, Pseudomonas aeruginosa, Neisseria gonorrhoeae*	Corneal scrapping
Bacterial endophthalmitis	*Staphylococcus aureus, Streptococcus pneumoniae, Enterobacterales*	Vitreous fluid aspirate

6.5.7.b Specimen collection of ocular infections

Specimen collection	Transport	If Delay is anticipated
Conjunctival swab: Obtain the specimen with a sterile, pre-moistened cotton or calcium alginate swab. Separate swab for both the eyes Immediately inoculate the material at the bedside onto Blood agar plate or chocolate agar plate	Immediately inoculate the material at bedside on Blood agar plate and Chocolate agar plate. Seal the culture plate with parafilm and transport the plates to the laboratory	Keep the plates at 37°C. Do not refrigerate
Corneal scraping: Using a Kimura spatula, gently scrape across the lower right tarsal conjunctiva. Smear the material in a circular area 1 cm in diameter on a clean glass slide. Prepare at least two slides.	Send the slides carefully wrapped and well labeled in a tamperproof container	Store in a tamperproof slide box
Bacterial endophthalmitis: Collect an aspirate of the vitreous fluid or perform a paracentesis of the anterior chamber using a needle aspiration technique to collect intraocular fluid.	After the aspiration of the sample, aspirate 0.5ml of media from sterile blood culture bottle. Apply luer lock to the syringe and send the sample as such	Inoculate cultures at the bedside by inoculating 1 or 2 drops of fluid onto Blood agar plate and Chocolate agar plate

6.5.8.a. Infections of the female genital tract

Syndrome	Organism suspected	Specimens which can be sent
Salpingitis (PID)	*Enterococcus sp., Escherichia coli, Hemophilus influenzae, Klebsiella sp., Listeria monocytogenes, Streptococcus agalactiae, Streptococcus pyogenes*	Culdocentesis laparoscopic sample of fallopian tubes and peritoneal cavity
Bartholinitis	*Escherichia coli, Hemophilus influenzae, Proteus mirabilis*	Aspirate from Bartholin gland
Cervicitis	*Streptococcus agalactiae*	Swab of endocervical canal

Endometritis/ Postpartum endomyometritis	*Enterococcus sp., Escherichia coli, Hemophilus influenzae, Klebsiella sp., Listeria monocytogenes, Streptococcus agalactiae, Streptococcus pyogenes*	Transvaginal aspirate of endometrium
Screening for Group B streptococcus	*Streptococcus agalactiae*	Low vaginal swab and Rectal swab
Vaginosis	*Gardnerella vaginalis, Mobiluncus, Mycoplasma hominis*	High vaginal swab

6.5.8.b Specimen Collection for infections of female genital tract

Specimen collection (Sterile leakproof universal container)	**If Delay is anticipated**
Bartholin cyst/ Culdocentesis/ fallopian tube: Aspirate from the duct/ Cul-de-sac/ fallopian tube	Send the sample within 2 hours of collection
Cervical swab: Insert the swab through endocervical canal, avoid touching the vaginal walls during collection	Send the swab sample in transport media
Endometrium: Insert endometrial suction curette or catheter-protected Dacron swab through cervical os and transfer beyond cervical opening into the uterine cavity, and collect the sample from within the cavity	Store at room temperature or at 4°C
High vaginal swab: Aspirate the fluid from vagina with sterile pipette or Dacron swab from posterior vaginal wall. Transport the swab in Amies or Stuart's medium	
Low vaginal and Rectal swab for Group B *Streptococcus* screening: Rayon or Dacron swabs, Fiber of flocked swabs with non-nutritive transport medium. Transport the swab in Amies or Stuart's medium.	Refrigerate at 4°C

6.5.9.a. Infections of the male genital tract

Syndrome	**Organism suspected**	**Specimens which can be sent**
Epididymitis	*Enterobacteriaceae, Pseudomonas sp.*	Swab or fluid from epididymis
Urethritis	*Hemophilus influenzae, Hemophilus parainfluenzae,*	Swab or exudate from urethra
Prostatitis	*Escherichia coli, Enterobacteriaceae, Enterococcus sp., Pseudomonas aeruginosa*	Swab or aspirate through Urethra
Prostrate abscess	*Staphylococcus aureus*	Abscess fluid or aspirate
Orchitis	*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococci, Streptococci*	Swab or fluid from testicles
Pyospermia	*Streptococcus pyogenes, Streptococcus pneumoniae, Escherichia coli, Staphylococcus aureus, Streptococcus agalactiae*	Semen sample

6.5.9.b Specimen collection for infections of male genital tract

Specimen collection (sterile leakproof container)	If delay is anticipated
Epididymis or testicular fluid: Aspirate the material from epididymis or testicles. Also collect a first voided and midstream urine sample	Store at room temperature or at 4°C
Semen sample: Sample is collected in sterile container after proper instruction to the patient. Following liquefaction, two swabs are dipped in semen sample and placed in Amies transport medium. One drop of semen is smeared on glass slide and allowed to dry	Do NOT refrigerate Send both the swabs in transport media along with the slide

6.6. Specimens for anaerobic culture

S. No.	Site of infection	Appropriate specimen	Method of Collection
1.	Head and Neck	Aspirate, Tissue Biopsy	Percutaneous needle aspiration surgically obtained
2.	Periodontal	Subgingival pocket plaque Aspirate	Paper points or scaler into transport medium by needle aspiration
3.	Pulmonary	Lung aspirate Tissue Biopsy, Deep bronchial secretions	• Percutaneous needle aspiration of lung • Transtracheal aspirate or protected bronchial brush
4.	Joint	Synovial fluid	Needle aspirate obtained at surgery
5.	Abdominal	Peritoneal Fluid Abscess contents Bile Tissue biopsy	• Needle aspirate obtained at surgery • Aspirate obtained at surgery or under CT or USG (avoiding contamination with bowel contents) • Bile obtained at surgery • Surgically obtained tissue
5.	Female genital tract	Peritoneal Fluid Endometrial material Tissue biopsy	• Culdocentesis • Endometrial suction • Surgically obtained tissue
6.	Bone	Biopsy Aspirate	• Curetting or scrapings obtained surgically • Aspirate of deep tissue via uninvolved skin surface
7.	Other Soft Tissue	Tissue biopsy Aspirate	• Surgically obtained tissue • Percutaneous needle aspiration • Tissue curetting
8.	Urine	Urine	Suprapubic aspirate

6.6.1 Specimen collection procedure for anaerobic organisms

Infectious process	Specimen	Procedure
Abscess	Aspirate	Aspirate material by needle and syringe through disinfected, uninvolved and intact tissue.

Sinus tracts, or deep draining wounds	Curetting	**1. Sinus tract:** Clean with 70% alcohol followed by iodine in circular motion moving outward in concentric circles 2cm beyond sinus tract. Irrigate with iodine preparation into the opening of sinus tract to disinfect the proximal area. Discard surface curetting and use deeper curetting. **2. Draining pus:** Aspirate material from the tract via flexible plastic catheter and syringe.
Oral or Gingival Abscess	Aspirate	**1. Oral cavity/Periodontal area:** Aspirate with aseptic precautions. **2. Supragingival plaque:** a) can be used to Gently scrape material with sterile scaler or sterile Gracey curette against root surface from the depth of the sulcus
Paranasal sinus secretions	Aspirate (from maxillary sinus)	Spray the nasal cavity below the anterior portion of inferior turbinate with 10% xylocaine and then swab the area to be punctured with cotton swab soaked in 2% tetracaine to anesthetize and disinfect the area. After 15-20 mins, aspirate material from maxillary antrum with a sterile 2mm diameter puncture needle attached to 20 ml syringe. Endoscopically obtained secretions (less satisfactory) can be collected with double-lumen catheter.
Superficial Ulcers	Curetting, Aspirate	Surface debridement to be done and then material from the base of ulcer is collected by curette or vigorous swabbing after discarding the superficial materials or curetting.
Female genital tract involvement	Peritoneal fluid, Intrauterine material (Endometrial, Uterine, Tubo-ovarian abscess) Tissue	1. Culdocentesis 2. Disinfect cervical os by wiping off excess mucus and swabbing with povidone-iodine. Insert protected sampling device for collection of intrauterine material. 3. The material from tubo-ovarian abscess or biopsy for tissue specimen should be collected at the time of surgery.
Specimens collected at the time of surgery	Tissue, Abscess material	Collect small portion of tissue (at least $5mm^3$ size) directly into anaerobic transport tube with agar plug in base.

6.7. Specimens for fungal culture

6.7.1. Blood culture: Refer 6.1

6.7.2. Cutaneous samples (Hair, skin, nails)

- Disinfect all sites with 70% alcohol
- **Hair:** hair root is most important part. Best collected by plucking. Infected area of the scalp can be scrapped with a sterile hairbrush/scalpel. 10-12 hairs should be submitted in a sterile container or envelope.
- **Skin:** Specimen should be collected from the edge of the lesion. Scrape with dull edge of a scalpel or glass slide. Transport in a clean envelope or between two clean glass slides taped together or sterile container. Sample quantity should be sufficient to be processed for both KOH mount and fungal culture.

- **Nails:** Clip or scrape with a scalpel. Material under nail should also be scrapped. Transport in a sterile container or envelope.

6.7.3. Sputum

- Collect an early morning prior to eating. Use mouth rinse and brush before collection specimen on two consecutive days.
- Collect 5-15 mL in a sterile container.

6.7.4. Other specimens: Other samples such as urine, CSF, sterile body fluids, pus etc. to be collected as for aerobic culture and serum sample in Yellow/ Red cap Vacutainers for serological tests.

6.8. Specimens for Mycobacteria culture

6.8.1. Sputum: Patient should be given clear instructions on how to collect sputum sample. One spot sample and one next day morning sample should be collected. **At least two or more sputum samples should be taken.**

6.8.1a. For spot collection of sputum samples

- Sputum collection cubicles should be in open crowd free space as air dilutes the aerosols.
- Sample should be collected in sterile, wide-mouthed, leak proof container with tight lid.
- Take a deep breath and hold the breath for 5 seconds. Slowly breathe out. Take another deep breath and cough hard until some sputum comes up into your mouth.
- Spit the sputum into the plastic cup. Keep doing this until the sputum reaches the 5 ml line (or more) on the plastic cup. This is about 1 teaspoon of sputum.
- Screw the cap on the cup tightly so it doesn't leak.
- Write the date you collected the sputum on the cap.

6.8.1b. Instructions for next day morning samples of sputum

- As soon as the patient wakes up in the morning (before he eats or drinks anything), instruct the patient to brush their teeth and rinse their mouth with water. Mouthwash should not be used.
- Patient should preferably go outside or open a window before collecting the sample.
- This helps protect other people from TB germs when he coughs.
- Put the cup into the box or bag given by the nurse.
- The cup should be given to the clinic or nurse within 2 hours.

6.8.2 Specimens other than sputum

- Refer to points 9.1-9.5 for instructions to collect all other specimen types.
- **Urine :** three consecutive early morning, voided midstream specimen should be collected. Minimum of 100 ml is ideal. 24 hours urine is not recommended.

- **Broncho-alveolar lavage/bronchial washings:** contamination of bronchoscope with tap-water (which may contain environmental Mycobacterium species) should be avoided. At least 5-10 ml should be collected.
- For fluids that may clot, sterile potassium oxalate (0.01- 0.02 ml of 10% neutral oxalate per ml fluid) or heparin (0.2 mg per ml) should be added.
- **Aseptically collected tissues/ endometrial biopsy**: collected aseptically in sterile containers with normal saline and without any fixatives or preservatives.
- **Gastric washings**: usually used for children where there are problems obtaining sputum. Collect samples early in the morning (before breakfast) on 3 consecutive days. Preferably, a minimum volume of 5 ml should be provided.
- **Blood is not an acceptable sample.**
- Swabs are suboptimal for recovery of mycobacteria due to limited material and the hydrophobicity of the mycobacterial cell envelope (often compromises a transfer from swabs onto media). **Dry swabs are unacceptable.**

6.9. Specimens for water bacteriology

6.9.1 Water from drinking water coolers/ tap

- Collect Sterile Glass Medical Flat Bottle (120ml approximately) with washer cap from room no. 124A (Enterobacteriaceae lab, Department of Microbiology, MAMC) on the day of sampling and inform the lab regarding number of samples that will be submitted.
- DO NOT open the cap of bottle until ready to collect water sample.
- Reach required site where water cooler is placed.
- Wipe tap nozzle with alcohol or spirit and allow to dry.
- Allow water to run for 1-2 minutes to wash off contaminating build-up in the tap.
- Each narrow-mouthed glass bottle is sterilized by hot air oven and has been provided with washer attached cap.
- Ensured that the mouth and inner side of the cap is neither touched by bare hands nor does it come in contact with any surface.
- Open the bottle cap and allow water to run into the bottle; bottle should not touch the tap.
- Collect only one sample for each drinking water cooler.
- 100ml water sample has to be collected in each bottle. (Total capacity of bottle =120ml approx.)
- Close the cap tightly and transport the bottles to the Room no 124A (Enterobacteriaceae lab, Department of Microbiology, MAMC).
- Do not tilt bottle to avoid leakage.
- Provide completely filled requisition form with the following information:
  - ✓ Site of water collection (exact detail in case of multiple samples, eg- water cooler 1st floor, water cooler PG room, etc)
  - ✓ Number of samples collected (total)

- ✓ Date & time of Sample collection
- ✓ Sample collected by
- ✓ Sample transported by

7.0. Transportation and storage of specimens

7.1. General instructions for transportation

- For culture, if direct inoculation is not possible, samples are transported to the laboratory as soon as possible.
- For specimen transported in a transport medium organism remains alive for 24 hours at room temperature.
- The blood/serum sample tube/vial, which is being transported, should not have cracks/leakage. It should preferably be made of plastic and be screw capped.
- The outside of the tube should be checked for any visible contamination with blood which should be cleaned.
- All the specimen vials must be adequately labeled with patient's details.
- The request slip should accompany the specimen and it should be placed in a separate place outside the transport box to prevent smudging on account of spillage.
- Person who transports specimens must be trained in safe handling practices and in decontamination procedures in case of a spill.
- Gloves should be worn when removing specimens from the primary container and for all manipulations of the primary container.

7.2. Specific Instructions for transport and storage

A. Blood culture

- Do not refrigerate blood cultures. Generally, hold at room temperature until processed, for a maximum of 4 h.
- In case of a delay in transportation, the inoculated bottle should be incubated at 37°C at the hospital and transported the following day. The microbiologist should be informed about the prior incubation.
- Blood cultures are transported at room temperature.
- Do not refrigerate blood cultures if there is a delay in transporting to laboratory.

B. Serum/plasma

- Transport within two hours at room temperature, or hold at 2-8°C if a delay is expected.
- Place the blood/serum sample in a thermocol/cardboard/steel box containing sufficient packing material, such as cotton gauze to absorb all the blood, and ice packs to maintain 2-8°C if delayed.
- Store blood/serum samples tightly capped in the refrigerator at temperature 2°-8°C for up to 3 days. Do not freeze the sample. These blood samples may also be stored at -20° C if to be used beyond 3 days.

C. Urine

- Samples are cultured as early as possible after collection, preferably within 2 hours.

- In case of delay, refrigerate at 4^0C for upto 24h, during holding period and transport. **DO NOT FREEZE**.
- If refrigeration is not possible and specimens are delayed in transport, collect in transport tubes with preservatives.
  - ✓ Examples: 0.5ml of freeze-dried boric acid-glycerol or boric acid-sodium.
  - ✓ Place at least 3 ml of urine into the transport tube to avoid an inhibiting or diluting effect on the microorganisms.

D. Cerebrospinal fluid

- Submit to laboratory as soon as possible and alert the laboratory that the specimen is in transit.
- Do not refrigerate specimen until after microscopy and culture have been performed.
- Time between collection to microscopy and culture should occur within a maximum of 2 hours.
- In cases where fungal/tubercular cultures or viral PCR of CSF are required, please ensure that the microbiologist is contacted in advance to facilitate timely processing of the sample.

E. Stool specimen

- In case of delay of more than 2 hours, a small amount of the stool specimen (together with mucus, blood and pus, if present) should be collected on two or three swabs and placed in a container with transport medium (Cary–Blair, Stuart or Amies).
- For cholera alkaline peptone water may be used.
- Store at 2-8°C.

F. Specimen for anaerobic culture

- Transport sample as soon as possible, in transport medium such as Robertson's cooked meat broth, and HI culture Transport medium (Thioglycolate medium in Polypropylene tube). This can be asked in advance from department.
- If delay is anticipated, the sample in transport medium can be kept for up to 48 hrs. at room temperature/ 37°C. Do not refrigerate.
- Aspirated material should be transported in transport medium and transportation in syringe should be avoided.

G. Specimen for mycobacterial culture

- If a delay is unavoidable, the specimens should be refrigerated.
- If specimens have to be transported at ambient temperatures, chemical preservation may be used. Mixing the fresh specimen with an equal volume of 1% cetyl pyridinium chloride in 2% sodium chloride.

8.0. Rejection of specimens

8.1. General Rejection Criteria

- Any mismatch of patient's particulars between the TRF and label on the sample.
- TRF with illegible writing or soiled with blood, fluid, urine etc.
- Samples in open tubes.
- Samples in broken tubes/ leaking container/ visibly soiled container.
- Specimen of inadequate quantity to permit complete examination.
- Samples without a requisition form or samples with insufficient data in the TRF making them untraceable.
- Swab specimens are rejected where frank pus/sterile fluids/ET aspirate is available.
- Tissue samples received in formalin.
- Haemolyzed or lipaemic blood sample.
- Tissue samples sent in formalin
- Samples received in non-sterile or inappropriate container.
- Male genital samples without transport medium.

8.2. Rejection criteria for culture

Specimen	Rejection criteria
Urine	Collected from the drainage bag in catheterised patients. Received after more than 2 hours of collection. Foley's catheter tip. Sample grossly contaminated with stool.
Blood or CSF	The barcode on the blood culture bottle should be clear and not covered with the patient's label. Sample received in red vial.
Sputum/ET	Saliva
Bronchoscopy samples	Sample contaminated with excess of blood
Sterile body fluids	Drainage tube specimens
Ocular	Swab samples directly sent to the laboratory are discouraged. Conjunctival swabs are rejected. Aspirate should be sent along with the conjunctival swab.
Specimens for anaerobic culture	• Specimens which are contaminated with normal flora (such as throat, nasopharyngeal, open wound, Vaginal, Cervical, Gingival or any other intraoral surface swabs, sputum), Bronchial washings or any specimen by bronchoscope, Gastric and small bowel contents (except for Blind loop or bacterial overgrowth syndrome), Large bowel contents (except for *C. difficile, C. botulinum.*), Ileostomy, colostomy effluents, stool. Urine, surface swabs from decubitus ulcers, perirectal abscess, foot ulcers, wounds, sinuses or sinus tracts • Samples not sent in appropriate anaerobic transport media.
Direct microscopy samples	Swabs for Ziehl Neelsen staining. Dry swabs for Gram staining.
Respiratory PCR	Cotton swabs with wooden sticks Swabs sent without VTM Samples not received in Cold chain.

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