Sample Collection and Transport

For

Culture & Sensitivity

Timing for receipt of samples:

On Working days	**:**	**9:00 a.m. to 3.00 p.m. (Department of Microbiology)**
		3.00 p.m. onwards to 8 a.m. (Emergency Laboratory)
On Holiday	**:**	**24 hrs Emergency Laboratory**

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1. BLOOD

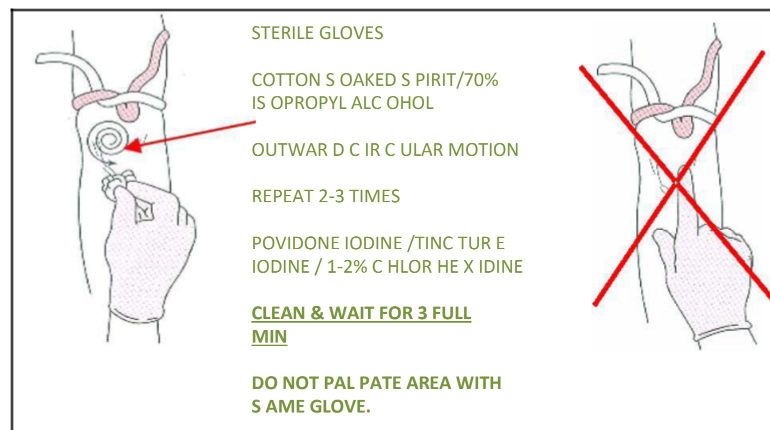
1.1. Collection and transport

To reduce blood culture contamination rate, collection may be improved by taking the following precautions.

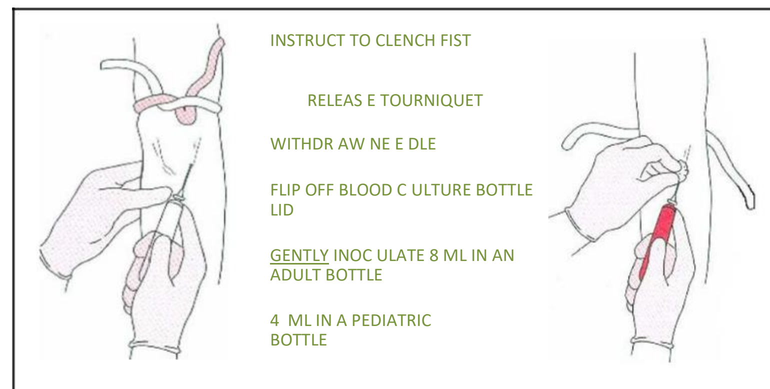
1.1.1. Prepare the site

- Select the site of venipuncture. If the patient is unusually dirty, wash the intended site with soap and water prior to venipuncture.
- Apply a tourniquet, 3-4 inches above the intended site of venipuncture. Alternatively, this can be done after cleaning.
- Put on examination gloves.
- 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.
- Allow to dry.

- Swab or wipe concentric circles of 2% w/v chlorhexidine with 70% isopropyl alcohol or 10% w/v povidone iodine/tincture of iodine, in a similar manner as given earlier- beginning in the center and continuing in an outward direction circularly for an area of 4 to 5 inches in diameter.
- Allow the povidone iodine to dry (2 minutes). For chlorhexidine gluconate (2%w/v)/ tincture I_2 (10%w/v), drying period is ~ 30 seconds.
- **Do NOT** touch the site after cleaning.
- Instruct patient to clench and unclench the fist.



- Perform phlebotomy using the needle and syringe.
- Release the tourniquet and withdraw the needle.
- Apply pressure to the site of venipuncture with a sterile cotton ball and place a Bandaid or bandage if necessary.



- Skin preparation with either alcohol, alcoholic chlorhexidine (2% w/v), or tincture of iodine (10% w/v) leads to lower blood culture contamination rates than does the use of povidone-iodine.

- **For pediatric patients**

< **2months:** Omit the iodine step, and clean two additional times with separate preparation pads saturated with 70% isopropyl alcohol or ethyl alcohol

> **2 months:** Chlorhexidine gluconate as a skin antiseptic is approved for use in pediatric patients two months of age and older.

1.1.2. Prepare the bottle

Prepare the septum of the blood culture bottle and the rubber stoppers on bottles or tubes.

Label the bottles with the patient's name and the date and time of draw.

Site of draw may be listed.

Note: In particular, please mention whether blood is collected from a central line or from peripheral venipuncture.

Collection through an intravenous line

- It is not necessary to discard the initial volume of blood or flush the line with saline to eliminate residual heparin or other anticoagulants.
- Vigorously wipe septa with 70% alcohol and allow drying completely, for 30 to 60 seconds.
- Pediatric bottles should not to be used for adult patients except for those elderly patients in whom it's difficult to obtain larger amounts of blood.

Table 2.1: Recommended total volume and numbers of blood cultures

Age & body weight	Amount (divided between 2 blood cultures)	Remarks
Neonates to 1 year (<4 kg)	0.5 to 1.5 ml	At least 1 ml Two separate venipunctures are generally, not possible
Children (< 40 kg)	10 to 20 ml	Blood culture volumes should be limited to <1% of total blood volume (Usually about 0.7 ml/kg). e.g. total sample limit would be 7 ml for a 10 kg patient and 28 ml for a 40 kg patient.
Adults & children (>40 kg)	30 to 40 ml	At least 10-20 ml of blood

- **Adult patient (50 kg):** 10-20 ml, divided between **two** blood cultures from **separate**, peripheral venipuncture sites.
- **Pediatric patient:** 6-10 ml, divided between two blood cultures.
- Initially obtain three blood culture sets within a 30-minute period before administration of empiric antimicrobial agents from patients presenting withpossible infective endocarditis. If those sets are negative at 24 hours, obtain two more sets of cultures, for a total of five sets overall.

1.1.3. Timing of blood cultures

Note:

- Ensure blood is collected before administration of antimicrobials
- Drawing blood before or during the fever spike is optimal for recovery of infective agents.
- Volume is more important **than** timing
- Thoroughly mix bottles to avoid clotting.

After phlebotomy, remove residual tincture of iodine from the patient's skin by cleansing with alcohol to avoid skin irritation.

Manual blood culture inoculation

For conventional blood culture method, blood culture for bacterial infections should be carried out in two bottles containing 50 ml each of tryptone soya broth and bile broth. After removing the kraft paper, inoculate the blood culture bottles.

1.1.4. Transport of blood culture bottles

In case of delay between collection and processing, **never refrigerate the bottle**. Preferably keep the bottle in a 35ºC incubator (available in emergency lab). Otherwise, leave the bottle at room temperature till transport to laboratory.

2. CSF

2.1. Collection and transport

Purpose: To identify the organisms causing pyogenic meningitis.

Note: This is an emergency procedure. The samples should be processed and reported immediately. The results of the smear should be informed to the concerned clinician and documented in the critical alert register.

2.1.1. Specimen collection

2.1.1.1. Lumbar puncture

- Cap, face mask, gown and gloves for physician drawing CSF are useful adjuncts to infection prevention. Disinfect the puncture site with antiseptic solution and alcohol in a manner identical to phlebotomy skin preparation for blood culture to prevent specimen contamination and introduction of infection.
- Insert a needle with stylet at the L3-L4, L4-L5, or L5-S1 interspace. When the subarachnoid space is reached, remove the stylet; spinal fluid will appear in the needle hub.
- Measure the hydrostatic pressure with a manometer.
- **Note:** Lumbar puncture opening pressure should not be considered a reliable measure of intracranial pressure in children.
- Collect the CSF into five calibrated sterile labeled tubes.
- Sequentially collect 2.0 ml of CSF each into three sterile calibrated tubes if only routine chemistry (total protein and glucose), bacteriology (culture & susceptibility), and hematology (cell count) are required.

2.1.1.2. Ventricular shunt fluid

- Clean the reservoir site with antiseptic solution and alcohol prior to removal of fluid to prevent introduction of infection.
- Remove fluid by aspiration of CSF from the Ommaya reservoir or by collection from the ventricular drain or shunt.
- Collect CSF into a minimum of three sterile calibrated tubes if only routine chemistry (total protein and glucose, tube no. 1), bacteriology (culture & susceptibility, tube no. 2), and hematology (cell count, tube no. 3) are required.

- An initial CSF sample should be collected prior to antimicrobial therapy for highest diagnostic sensitivity.

2.1.2. Specimen transport

- NEVER refrigerate.
- Each sterile calibrated tube containing CSF must be properly labelled with the patient's name, unique identification number, and the date & time of collection.
- Requisition must be complete with demographic and specimen collection information. Record clinical data for proper processing of specimen.
- Submit to laboratory as soon as possible and alert laboratory that specimen is in transit.

2.1.3. Rejection criteria

- Call physician to prioritize requests if there is insufficient volume.
- Specimens in leaky containers must be processed, but alert the physician of the possibility of contamination.

3. BODY FLUIDS FROM STERILE SITES

3.1. Specimen collection

- Body fluids from sterile sites should be collected by percutaneous aspiration for pleural, pericardial, peritoneal, amniotic, and synovial fluids.
- Use care to avoid contamination with commensal microbiota.
- Clean the needle puncture site with alcohol, and disinfect it with an iodine solution [1-2% tincture of iodine or a 10% solution of povidone iodine (1% free iodine)] to prevent specimen contamination or infection of patient (if tincture of iodine is used, remove with 70% ethanol after the procedure to avoid burn).
- Aseptically perform percutaneous aspiration with syringe and needle to obtain pleural, pericardial, peritoneal, or synovial fluid. Use safety devices to protect from needle exposure.
- Immediately place a portion of the joint fluid or peritoneal fluid collected from patients with CAPD or SBP into aerobic and anaerobic blood culture bottles, retaining some (0.5 ml) in syringe for Gram stain and direct plating.
- Use the minimum and maximum volumes recommended by the bottle manufacturer (generally up to 10 ml is the maximum for each bottle).
- Alternatively, inoculate the blood culture bottles after receipt in the laboratory.
- Submit other fluids and the remainder of specimens after inoculation of blood culture bottles in one of the following: a sterile, gassed-out tube or a sterile blood collection tube without preservative; however, fluids in such tubes may clot during transport.

3.2 Specimen transport

- Submit to laboratory as soon as possible and, if from a normally sterile site, alert laboratory that specimen has been submitted.
- **Do not** refrigerate.
- Label specimens with patient demographics and date, time, and site of collection,*e.g.*, left knee joint fluid.
- Record the patient diagnosis for improved processing of specimen.

4. OCULAR SPECIMENS

4.1. Specimen collection and transport

Note: Most eye specimens should be collected by an ophthalmologist. These specimens should be inoculated onto culture media at the bedside, in the clinic or the physician's office. A variety of techniques are used to collect material from different parts of the eye. The conjunctiva is constantly contaminated by various bacteria from the environment and ocular adnexa. Therefore, specimens from the conjunctiva serve as a control when compared with specimens collected by more aggressive or invasive techniques.

Considerations

- Provide fresh media to the clinical areas routinely collecting ocular cultures, and once collected immediately transport inoculated media and slides to the laboratory.
- Obtain viral and chlamydial samples before topical anesthetics are instilled.
- Obtain samples for chlamydial cultures with calcium alginate swabs.
- **Note:** Calcium alginate swabs may be toxic for *Neisseria gonorrhoeae* (for which rayon or cotton swabs could be used).
- For viral cultures, use Dacron or cotton swabs with non-wood shafts.

4.1.1. Collection by anatomic site

4.1.1.1. Conjunctiva (bacterial conjunctivitis) and lid margin (blepharoconjunctivitis)

- Obtain the specimen with a sterile, pre-moistened cotton or calcium alginate swab.
- Roll the calcium alginate or cotton swab over the conjunctiva before topical medications are applied.
- Culture both eyes with separate swabs.
- Immediately inoculate the material at the bedside onto BAP and CHOC.
- Inoculate the swab from the right conjunctiva in horizontal streaks, and inoculate the swab from the left conjunctiva in vertical streaks, each on one half of the same agar plate.
- Inoculate specimens from the right and left lid margins, if collected, by making an R and an L to represent the respective sites on another agar plate.
- Obtain conjunctival scrapings for a smear preparation as follows:

- Instill 1 or 2 drops of proparacaine hydrochloride.
- 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.
- Immerse the slides in 95% methyl alcohol or 95% methanol for 5 minutes.
- Repeat steps for the left conjunctiva.

4.1.1.2. Cornea (bacterial keratitis)

- Instill 1 or 2 drops of proparacaine hydrochloride (local anesthetic for ophthalmic instillation).
- Obtain conjunctival samples as described above, and then obtain corneal scrapings from the advancing edge of the ulcer by scraping multiple areas of ulceration and suppuration with a sterile Kimura spatula, using short, firm strokes in one direction (keep the eyelid open, and be careful not to touch the eyelashes).
- Obtain approximately three to five scrapings per cornea.
- Inoculate each set of scrapings onto BAP and CHOC, using a 'C' formation for each scraping.
- Prepare smears by applying the scrapings in a gentle circular motion over a clean glass slide or by compressing material between two clean glass slides and pulling the slides apart.

4. RESPIRATORY SPECIMENS

Purpose: To isolate and identify the potentially pathogenic organisms from upper and lower respiratory tracts (URT and LRT) aiding in the diagnosis of infections.

Sputum cultures are done primarily to identify the pathogens that cause pneumonia or bronchopneumonia: community-acquired or hospital-acquired.

5.1. Specimen collection and transport

5.1.1. Sputum

- Spontaneous: Early morning specimen generated after a bout of cough.

- Having the patient brush his or her teeth and gargle with water immediately before obtaining the sputum specimen reduces the number of contaminating oropharyngeal bacteria.
- Collect specimen resulting from deep cough in a sterile screw-cap cup or other suitable sterile collection assembly of about 100 ml capacity.
- To prevent contamination of the outside of the container, the patient should be instructed to press the rim of the container under the lower lip to catch the entire expectorated cough sample.
- Tightly screw on the cap of the container. Wipe off any spilled material on its outside with a tissue moistened with disinfectant, but take care not to let any disinfectant enter the container. Such communication with patients can be rewarding. In addition, patients should remove dentures during the specimen collection.
- Early-morning sputum samples should be obtained because they contain pooled overnight secretions in which pathogenic bacteria are more likely to be concentrated. Twenty-four-hour collections should be discouraged
- Deliver the specimen to the laboratory as quickly as possible, preferably within 2 hours, for delicate bacterial, viral and mycoplasma pathogens may die out during longer delay.

5.1.2. Endotracheal aspirate (ETA)

- Endotracheal aspiration should be done with a sterile technique using a 22 inch, 12F suction catheter. The catheter should be introduced through the endotracheal tube for at least 30 cm. Gentle aspiration is then performed without instilling saline solution. The first aspirate is discarded.
- The second aspirate should be collected after tracheal instillation of 5 ml saline in a mucus collection tube. [If very little secretion is produced by the patient, chest vibration or percussion for 10 minutes should be used to increase the retrieved volume ($\geq$ 1 ml)].
- The specimens should be sent to laboratory and cultured within 1 hour of ollection.

5.1.3. Bronchoalveolar lavage (BAL)

In this procedure 120 ml of saline should be infused into a lung segment through the bronchoscope to obtain cells and protein of the pulmonary interstitium and alveolar spaces. Send a portion of it to the laboratory.

5.1.4. Sinus aspirate

Collection of specimens from patients with sinusitis should be performed by otolaryngologists who perform nasal endoscopy or sinus puncture and aspiration.

5.2. Type of container

Collect in a sterile leak proof screw-cap container.

5.3. Rejection criteria

5.3.1. For sputum and endotracheal aspirate specimens

- Duplicate specimens received on the same day unless the initial sample was inappropriate for culture according to microscopic evaluation.
- Repeat cultures at intervals of less than every 48 hours will not be accepted.
- Reject the following specimens for diagnosis of lower respiratory tract disease:
  - 24 hours sputum collection
  - Contaminated sputum and endotracheal specimens as per Gram stain rejection criteria (see below)
  - Specimens that are visually saliva only
  - Specimens that are visibly contaminated with toothpaste or other substances
  - Nasal washes or swabs of nares to diagnose sinusitis
- Sputum samples are highly contaminated with normal anaerobic flora of the upper respiratory tract. Therefore, anaerobic culture should not be done.

6. PUS

Purpose: To isolate and identify bacterial etiological agent(s) in deep-seated pus/wound specimens.

6.1 Specimen collection

- Preferably collect specimen prior to initiation of therapy and only from wounds that are clinically infected or deteriorating or that fail to heal over a long period.
- Cleanse surrounding skin or mucosal surfaces.
- For closed wounds and aspirates, disinfect with 2% chlorhexidine or 70% alcohol followed by an iodine solution [1 to 2% tincture of iodine or a 10% solution of povidone-iodine (1% free iodine)]. Remove iodine with alcohol prior to specimen collection.

- For open wounds, debride, if appropriate, and thoroughly rinse with sterile saline prior to collection. Sample viable infected tissue, rather than superficial debris.

6.1.1 Wound or abscess aspirates

- Samples collected by using a syringe and needle should be placed in a sterile container or blood collection tube without anticoagulant (*e.g.*, Vacutainer® or similar type) for submission to the laboratory.
- A portion of the sample should also be placed in a sterile tube containing anaerobic medium like RCM if an anaerobic culture is required.

6.1.2. Open wounds

- Cleanse the superficial area thoroughly with sterile saline, changing sponges with each application. Remove all superficial exudates.
- Remove overlying debris with scalpel and swabs or sponges.
- Collect biopsy or curette sample from base or advancing margin of lesion.

6.1.3. Pus

- Aspirate the deepest portion of the lesion or exudate with a syringe and needle.
- Collect a biopsy sample of the advancing margin or base of the infected lesion after excision and drainage.
- For bite wounds, aspirate pus from the wound, or obtain it at the time of incision, drainage, or debridement of infected wound.

6.1.4. Tissues and biopsy samples

- Tissue biopsy samples should be collected from areas within and adjacent to the area of infection. Large enough tissue samples should be collected to perform all of the tests required (i.e., 3 to 4 mm biopsy samples).
- If anaerobic culture is required, a separate piece of tissue should be submitted in a sterile tube containing anaerobic medium like RCM.
- Collect swabs only when tissue or aspirate cannot be obtained.
- Limit swab sampling to wounds that are clinically infected or those that are chronic and non-healing.
- Remove superficial debris by thorough irrigation and cleansing with non-bacteriostatic sterile saline. If wound is relatively dry, collect with two cotton-tipped swabs moistened with sterile saline.

- Gently roll swab over the surface of the wound approximately five times, focusing on area where there is evidence of pus or inflamed tissue.

Note: Organisms may not be distributed evenly in a burn wound, so sampling different areas of the burn is recommended. Blood cultures should be used to monitor patient status.

6.2. Standard precautions to be followed while handling the specimen

Note: Syringes with the needle attached is not acceptable due to the sharps and biohazard risk to staff.

- Grossly contaminated specimen or leaky containers and collection containers of doubtful sterility must be noted and mentioned.
- Deliver aspirates and tissues to the laboratory within 30 minutes for best recovery.
- Keep tissues moist to preserve organism viability.
- Do not refrigerate or incubate before or during transport. If there is a delay, keep sample at room temperature, because at lower temperature there is likely to be more dissolved oxygen, which could be detrimental to anaerobes.

6.3. Rejection criteria

- For anaerobic culture, avoid swab collection if aspirates or biopsy samples can be obtained**.**
- Do not accept specimens for microbiological analysis in container with formalin.

7. URINE

The most common urine specimen received is the per-urethral voided urine. Healthy urethra is unsterile and it is extremely critical that urine specimens be collected carefully to minimise urethral contamination. There are several types of urine specimens and the results of each type are determined by different guidelines. Therefore, it is essential that each urine specimen received by the laboratory is clearly labelled as to the type of collection of urine specimen.

7.1. Collection of urine

7.1.1. Midstream clean catch urine

- The midstream clean catch urine is the most common type of urine specimen.
- The technique involved in collection is based on voiding the first portion of urine, which is most likely to be contaminated by urethral commensals.
- It is recommended that the first voided morning specimen be collected, as bacteria would have multiplied to high levels after overnight incubation in the bladder.
- If not possible, the urine can be collected during the day, preferably 4 hours after the last void, keeping in mind that the counts may be lower, yet significant.
- Midstream clean catch urine should be collected in a sterile, wide mouth, screw capped bottle after very thorough preliminary cleaning of external genitalia with soap and water. Antiseptics should not be used for this purpose.

7.1.2. Indwelling catheter

- Hospitalized patients with indwelling catheter are especially at risk of developing UTI.
- To avoid contamination, the specimen should be collected by disinfecting a portion of the catheter tubing with alcohol & puncturing the tubing directly with a sterile syringe with needle and aspirating the urine.
- The urine MUST NOT be collected from the drainage bag.

7.1.3. Suprapubic collection

- The suprapubic collection avoids urethral contamination but is invasive.
- This procedure is usually reserved for infants and adults, from whom it is difficult to obtain a midstream clean catch urine specimen.
- Disinfect the skin above the bladder and plunge a sterile needle with syringe into the bladder; aspirate the urine and transfer to a sterile container.

7.1.4. Percutaneous nephrostomy (PCN) aspirate

- Percutaneous nephrostomy aspirate is urine collected directly from renal pelvis.
- If the sample is a PCN catheter sample, collection must be done as explained for indwelling catheters and not from the drainage bag.

7.1.5. Cystoscopy specimens

- Cystoscopy specimen is urine collected from the bladder during cystoscopy.

7.1.6. Ileal conduit specimen

- Ileal conduit specimen is collected after cleaning stoma site.
- A fresh drain of urine is collected. It must not be collected from the urine drainage bag.

7.1.7. Intermittent catheter specimen

- A red rubber catheter should be introduced into the urethra periodically to drain urine from the bladder.
- It should be collected directly into a specimen container.

7.2. Specimen Transport

- In case of delay, it may be refrigerated up to a maximum of 24 hours before plating.
- Urine must be transported to the lab as soon as possible to be cultured immediately within 2 hrs.

8. FAECAL SPECIMENS

8.1 Specimen collection and transport

- A small quantity of solid/semisolid stool or one third of the container in case of watery stool is collected in a sterile screw-capped disposable 40 ml container.
- A rectal swab is not recommended as the material obtained is never adequate for all the tests or for inoculating all the media used for culture.
- The sample should be collected preferably prior to initiation of antibiotics in the container directly, taking care not to soil the outside of the container. Samples should not be collected from bedpan.
- The sample should be immediately transported to the laboratory on collection.