1. Introduction

This document lays down standard operational procedures (SOP) for the processes involved in the collection of samples from recruited subjects at the AFI study sites.

2. Objective

This SOP describes the step-by-step method to obtain clinical specimens from patients enrolled in the AFI study, for laboratory testing with proper specimen identification and handling, while ensuring patient and staff safety.

3. Responsibilities:

Study Laboratory technician/Research assistant is responsible to read and collect the samples according to this document.

4. Process:

4.1. General instructions:

- Identify the AFI case (MCVR SOP F 01)
- Obtain informed consent (MCVR SOP F 02)
- Collect all the specimens as described below for Acute febrile illness (AFI)
- Label all the samples properly and also fill the sample detail forms properly (mention as Acute, Discharge or Follow up).
- If it’s not possible to collect the sufficient amount of blood from the patient as per the study protocol, priority is for Serum.

4.2. Specimens to be collected at the time of recruitment:

- Blood in plain vacutainer for serum (4ml, Children-2ml)
- Blood for culture (Adults-5ml, Children-2ml)
- A throat swab in Viral transport media (VTM)
- 1-2 ml of urine (2 containers)
- Blood in EDTA tube (1-2 ml)

4.3. Additional samples to be collected:

- In case of AFI with diarrhea, collect stool sample
- In case of AFI with rash collect vesicle swab (one in VTM & one moist)
- In case of AFI with parotitis collect saliva
- In case of Acute Encephalitis Syndrome (AES), if Cerebrospinal Fluid (CSF) was collected as a part of case management whenever physician performs lumbar puncture, CSF will be included in the study
- In case of suspected black eschar, collect swab from the lesion or exfoliated skin.
- In case of any other clinical manifestations, case has to be discussed with principle investigator.
4.4. Specimens to be collected at the time of Discharge

- Blood (3-5 ml).

4.5. At the time of Follow up:

- Blood (3-5 ml)

Procedure for collection of blood

5. Introduction:

Serum is the liquid fraction of whole blood that is collected after the blood is allowed to clot. Serum can be used to do number of diagnostic tests like ELISA, rapid diagnostic tests, immunoserological tests and PCR for the detection of both antigen and antibodies to numerous pathogens causing infectious diseases. Both acute, discharge and follow-up samples must be collected to permit a definitive diagnosis.

Blood cultures are collected from patients with suspected sepsis or bacteremia. Virtually any organism may cause bacteremia. Thus, the isolation of all organisms from a blood culture must be considered significant and correlated with the clinical picture. Blood cultures should be collected from a patient with suspected bacteremia prior to the initiation of antimicrobial therapy.

EDTA Blood sample is collected during acute phase from all patients identified as AFI case.

6. Requirements:

- Disposable gloves
- Alcohol swabs, isopropyl alcohol
- Tourniquet
- Specimen Tubes:
  - EDTA Blood - Vacutainer specimen tubes (Tubes with Violet or lavender colored tops)
  - Plain blood - Vacutainer specimen tubes (Tubes with red colored tops)
  - BacT/Alert Blood culture bottles
- Disposable syringes
- Cotton balls
- Sharps disposal/ puncture resistant container containing hypochlorite solution
- Markers
- Refrigerator
7. Procedure:

7.1 Patient preparation:

- Identify the patient and explain the procedure to the patient.
- Make the patient comfortable and gain the patient’s cooperation.
- Position the patient. The patient should sit in a chair, lie down or sit up in bed. Hyperextend the patient’s arm.

7.2 Skin preparation:

- Before skin preparation wash your hands with soap and water.
- Wear the sterile pair of gloves
- Cleanse the venipuncture site with 70% isopropyl alcohol.
- Cleanse in a circular fashion, beginning at the site and working outward.
- Allow the site to dry.
- Do not touch the venipuncture site after skin preparation.

7.3. Plain blood and EDTA Blood Collection:

- All required materials for blood drawing should be assembled before performing the procedure.
- New sterile, single use needles and Vacutainer tubes are to be used for each blood draw.
- Vacutainer tubes should be labelled by patient identification number, patient’s name, age, sex etc. before sample collection.
- Also write the details whether the sample is Acute, Discharge or Follow up sample.
- Prepare a 10ml syringe (5ml for pediatrics).
- Clean the site of venipuncture with an alcohol swab.
- Gently insert the needle (at a 15-30 degree angle with the surface of the arm) into the lumen of the vein. Avoid excessive probing and trauma to the site.
- Once enough blood has been withdrawn, undo the tourniquet with the needle still in place.
- Take cotton swab and place over site of needle insertion (venipuncture) and gently remove the needle.
- Apply direct pressure with the cotton swab over the puncture site to stop any bleeding. This should be carried out for 2mins, after which the swab should be removed to ensure bleeding has stopped.
- For EDTA Blood - Transfer blood into labelled vacutainer (Tubes with Violet or lavender colored tops). Mix by inverting tubes 6-8 times.
- For plain blood - Transfer blood into labelled vacutainer (Tubes with red coloured tops), allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 15-30 minutes.
- Mix by inverting tubes 6-8 times.
- Samples after collection must be stored at 2-8°C.
After completion needles must be properly disposed of in a puncture resistant container containing hypochlorite solution. They are never to be cleaned and reused for any purpose.

DO NOT recap or bend the needle.

Samples after collection must be stored at 2-8ºC. (MCVR SOP F 04)

After completion needles must be properly disposed of in a puncture resistant container containing hypochlorite solution. They are never to be cleaned and reused for any purpose.

DO NOT recap or bend the needle.

7.4. BacT/Alert –

7.4.1. Preparation of BacT/Alert bottles:

Prior to use, examine the bottles for evidence of damage or deterioration (discoloration).

Do not use a bottle containing media which exhibits turbidity or excess gas pressure, as these are signs of possible contamination.

Check the expiry date printed on each bottle. Discard bottles that have expired.

Mark BacT/Alert culture vial label(s) at desired fill level.

Remove flip-off caps from BacT/Alert culture vials(s).

Label the bottle with patient details.

DO NOT write anything on the Bar code label or do not damage the bar code label.

Wipe tops of vials with single alcohol swab and allow to dry. Do not use iodine as it may damage the septum.

7.4.2. Volume:

1. Children: 1 to 5 ml of blood per venipuncture.

2. Adult: 5 to 10 ml of blood per venipuncture.

7.4.3. Blood collection using needle and syringe:

Blood for culture should be drawn from veins, not arteries.

A 10 ml syringe with a 21 gauge needle is recommended but other sizes may be used.

Insert the needle into prepared vein and collect 10 ml blood in syringe.

Withdraw needle after collecting 10ml blood in syringe.

Transfer blood into labelled BacT/Alert bottle.

After collection the samples store the bottles at room temperature.

All swabs and cotton pieces are placed in yellow bags for disposal.

Discard used syringe and needle into discard bin containing hypochlorite solution.
Procedure for collection of Urine

8. Introduction:

Under normal circumstances urine is sterile. The lower part of the urethra and the genitalia are normally colonized by bacteria, many of which may also cause urinary tract infection. Urinary tract infection (UTI) results from the presence and multiplication of microorganisms, in one or more structures of the urinary tract, with associated tissue invasion. This can give rise to a wide variety of clinical syndromes. Fresh urine sample is collected during the acute phase of the febrile illness. The clinical information obtained from a urine specimen is influenced by the collection method, timing and handling. For microbiological examination urine must be collected as a “clean catch-mid-stream” specimen. In case of catheterized patients, collect the urine specimen directly from the catheter.

9. Requirements:
   • Clean, sterile wide mouth, screw-top specimen containers (50ml or more)
   • Disposable gloves
   • Gauze pads
   • Soap and clean water (or normal saline) if possible.

10. Procedure:
   • Explain procedure carefully to the patient as instructions increase likelihood of proper specimen collection.

11. Sample collection from female:
   • Wash hands thoroughly with soap and water. Dry with paper towel.
   • With one hand, spread genital skin folds apart.
   • Using soap and water, wash vulva, wiping from front to back.
   • Repeat, wiping from front to back.
   • Void first portion of urine into toilet.
   • Void midstream portion into sterile container. Do not touch the inside of the container.
   • Stop collection when container is about half full, complete void into the toilet.
   • Screw cap the container tightly.
   • Wash hands

12. Sample collection from male:
   • Wash hands thoroughly with soap and water. Dry with paper towel.
   • Pull back foreskin, if uncircumcised and cleanse glans penis with soap and water.
Void first portion of urine into the toilet and then void the midstream portion into the urine container. Do not touch the inside of the container.

Stop collection when container is about half full, complete void into the toilet.

Screw cap the container tightly.

Wash hands.

**Procedure for collection of respiratory tract specimens**

13. **Introduction:**

Specimens are collected from the upper or lower respiratory tract, depending on the site of infection. Upper respiratory tract pathogens (viral and bacterial) are found in throat and nasopharyngeal specimens. Respiratory tract samples are collected during the acute phase of illness. When acute epiglottitis is suspected, NO attempt should be made to take throat or pharyngeal specimens since these procedures may precipitate respiratory obstruction.

14. **Requirements:**

- Appropriate PPE (Gloves, masks, safety goggles, mask etc.)
- Viral Transport media
- Dacron and polyester swabs
- Tongue depressor
- Clean, sterile wide mouth, screw-top specimen containers (50ml or more)

15. **Procedure:**

- Explain the procedure carefully to the patient and make him/her comfortable.
- Wear appropriate PPE before collecting the sample.
- Label the specimen containers with patient details.

15.1. **Method of collecting a throat swab:**

- Do not use any mouth washes before the test
- Tilt the head back, open the mouth widely
- A tongue depressor can be used to press the tongue downward to floor of the mouth
- Rub a sterile swab over the whole of pharynx or tonsil
- Withdraw the swab without touching cheeks, teeth, tongue or gums
- Patient should try to resist gagging and closing the mouth while the swab touches the back of throat near the tonsils.
• Immediately inoculate the swab in vial of VTM (viral transport medium), close the lid and transport in cold chain (2-80°C) *(MCVR SOP F 04)*

**15.2. Method of collecting a nasopharyngeal swab:**
• Patient should sit with back of head against a wall
• Tilt the head back at a 70° angle
• Inert the swab into one nostril straight back (not upwards)
• Continue along the floor of nasal passage until resistance is met
• Rotate the swab gently for 5-10 seconds to loosen epithelial cells
• Immediately inoculate the swab into VTM (viral transport medium) close the lid and transport in cold chain (2-80°C) *(MCVR SOP F 04)*

**15.3. Method of collecting a nasal swab:**
• Ensure that patient does not blow his/her nose prior to taking the nasal swab
• Tilt the head back gently and steady the chin
• Insert cotton bud end of dry sterile swab into right nostril, rub firmly against the turbinate to ensure swab contains cells as well as mucus
• Immediately inoculate the swab into VTM (viral transport medium) close the lid and transport in cold chain (2-80°C). *(MCVR SOP F 04)*

**15.4. Method of collecting a saliva sample:**
• Ensure that the patient do not eat any food just before collecting the sample.
• Instruct the patient to allow saliva to pool in the mouth.
• Ask the patient to spit into a sterile wide mouth screw capped container.
• Screw cap the container tightly.
• Wipe the outside of the container neatly and transport in cold chain (2-80°C). *(MCVR SOP F 04)*
Procedure for collection of fecal specimen

16. Introduction:

Stool specimens are most useful for microbiological diagnosis if collected soon after onset of diarrhea i.e., during acute phase (for viruses < 48 hours and for bacteria < 4 days), and preferably before the initiation of antibiotic therapy. Stool is the preferred specimen for culture of bacterial, viral, and parasitic diarrheal pathogens. Rectal swabs showing faeces may also be used from infants. In general, rectal swabs are not recommended for the diagnosis of viruses.

17. Requirements:

- Clean, dry, leak-proof screw cap container or stool specimen collection container.
- Sterile cotton swabs
- Sterile gloves
- Marker pens

18. Procedure:

18.1. Method of collecting a stool specimen:

- Label the specimen container with the patient name and study number.
- Ask the patient to collect freshly passed stool in a clean bed pan or any other plastic container.
- Use the spoon attached to the cap of the container to place the specimen (5 ml liquid or 5 g solid), in a container. OR inoculate the sample into a Cary Blair medium.
- Instruct the patient not to fill the specimen container up to the brim.
- Instruct the patient to collect the sample which is unmixed with the urine.
- Close the lid tightly, seal it with a cellophane tape and transport. (MCVR SOP F 04)

18.2. Method of collecting a rectal swab:

- Moisten a swab in sterile saline.
- Insert the swab tip just past the anal sphincter and rotate gently.
- Withdraw the swab and examine to ensure that the cotton tip is stained with faecal material.
- Place the swab in sterile tube/container containing the appropriate bacterial or viral transport medium.
- Break off the top part of the stick without touching the tube and tighten the screw cap firmly. Label the specimen tube and transport. (MCVR SOP F 04)
Procedure for collection of Vesicle Swab

19. Introduction:

For most dermatological conditions, diagnosis may be established on the basis of physical examination of the skin lesions such as nature of the skin lesions (erythematous, macular, papular, maculopapular, vesicular, bullous, petechial, purpuric, etc.) and the anatomic distribution of spread (central, peripheral, diffuse, etc.). In cases of indeterminate diagnoses, collection of specimens from rashes and/or skin lesions may be necessary. In the case of vesicular rashes, specimens for microscopy and culture are taken directly from vesicles. In other exanthemata (macular and/or papular), the diagnosis may be more readily established from alternative specimens (e.g. blood cultures, serology).

20. Requirements:

- Sterile saline
- 70% alcohol
- Sterile swabs (Dacron/synthetic tipped plastic swab)
- VTM
- Sterile screw-cap vials
- Sterile lancets or needles (for piercing of vesicles)
- Syringe with wide-bore needle (for aspiration of abscesses/buboes)

21. Procedure

21.1. Collection of vesicle swab from vesicular or vesiculo-pustular rash (for diagnosis of viral infections):

- Label the VTM tube with patient name and study Id.
- Examine the body part and choose the vesicle (look for recently formed vesicle).
- Clean the surface using 70% alcohol and allow it to dry.
- Pierce/rupture the roof of fluid-containing vesicle with sterile lancet/hypodermic needle.
- A sterile swab is then used to vigorously swab the base of the lesion — applying enough pressure to collect epithelial cells without causing bleeding—and collect vesicular fluid.
- It is important to collect infected epithelial cells from the base of the lesion because they usually contain a significant amount of virus.
- Collect the sample in two swabs.
- Immediately place one of the swab directly into virus transport medium, cut the upper portion of the swab and close the lid tightly.
- Place the second swab in a sterile screw-cap vials.
- Specimens for virus isolation should be refrigerated at 4-8°C, and transported to the laboratory as rapidly as possible, maintaining the cold chain (2-8°C). (MCVR SOP F 04)

21.2. Aspiration of abscesses:
Disinfect the skin overlying the abscess/bubo with 70% isopropyl alcohol.
Aspirate the fluid from the abscess with a sterile needle and syringe.
Transfer the aspirate aseptically into a sterile tube with transport medium and transport to the laboratory as rapidly as possible, maintaining the cold chain (2-8°C).

Procedure for collection of specimens for suspected for black eschar

22. Introduction:
Cutaneous anthrax follows deposition of the organism onto a cut, sore or abrasion on the skin, occurring particularly on exposed areas of the hands, arms, or face. An area of local edema becomes a pruritic macule or papule, which enlarges and ulcerates after 1-2 days. Small, 1-3 mm vesicles may surround the ulcer. The vesicles may become hemorrhagic, with satellite vesicles. A painless, depressed, black eschar (necrotic ulcer) forms in 3-7 days. The tissue surrounding the skin lesion is often erythematous, and may have varying degrees of edema (brawny, gelatinous, non-pitting edema).

23. Requirements:
- Sterile saline
- 70% alcohol
- Sterile swabs (Dacron/synthetic tipped plastic swab)
- Sterile screw-cap vials
- Tissue paper/ aluminum foil
- Blood agar culture plates

24. Procedure - Collection of lesion swab/ exfoliated skin swab/ wound swab (for diagnosis of cutaneous anthrax):
- Label the 15ml tube with patient name and study Id.
- Examine the body part and choose the lesion or the exfoliated skin (look for recently formed eschar).
- Exfoliated skin/ lesion will be removed using a sterile swab.
- The swabbed material will be then placed in a tissue paper or aluminum foil, wrapped securely in a zip lock container and sent in triple layer packaging (MCVR SOP F 04)
Wound swab collection- Collect two swabs by rubbing and scraping the base of the wound with sterile swab.
One swab will be put into the 8-10 drops saline into a 15 ml tube
The second swab will be plated into the blood agar culture plate inside a Biosafety Cabinet Class II Type A2, while strictly following all the biosafety guidelines.