FIEBRE Standard Operating Procedure F.04			
Title	**Collection of Patient Samples on Day 0: Blood, Pharyngeal Swabs, and Urine**		
SOP Reference		***Version***	***Date of effect***
F.04		3.2.4	16 Apr 19

SOP Development

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Review Tracker

Due date for next review	Reviewer name	Signature	Date reviewed
31 July 2018	Kate Haigh		20 Nov 18
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Revision History

Version No.	Effective date	Reason for change
2.0	20 Aug 2018 [depends on site-specific ethics approval]	Adjust blood volumes as in TABLE at end of SOP
3.0	20 Nov 2018	To include oropharyngeal swabs
3.2.1	17 Dec 2018	Adjust blood volume split between EDTA and plain tubes in children weighing less than or equal to 7kg [all sites], and to include urine sample for LAM on adults who are HIV reactive [site-specific]
3.2.2	29 Jan 2019	Clarification that NP and OP swabs should both be stored in the same tube
3.2.3	03 Apr 2019	Clarification in table at end of SOP and in text that the prior mentioned extra 0.5ml of blood from those weighing ≤7kg is for storage as whole blood
3.2.4	16 Apr 2019	CrAg testing added to blood volume table appendix

SOP User Confirmation

I acknowledge that I have read, understood and agree to follow this SOP

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1 Title: COLLECTION OF PATIENT SAMPLES ON DAY 0: BLOOD, PHARYNGEAL SWABS, AND URINE

2 Purpose:

To describe the procedures for collecting venous blood, pharyngeal swabs and urine from newly enrolled patients for the FIEBRE study

3 Responsible staff:

[Site-specific]

4 Background & Rationale:

On the day of patient enrolment, a venous blood sample and a naso- and oropharyngeal (NP and OP) swab will be collected from each patient for study laboratory procedures. A urine sample will be collected from young children, and from patients of any age who have symptoms of possible urinary tract infection. In addition, in Malawi, Mozambique and Zimbabwe, a urine sample will be collected from adult participants (>/= 15 years) who are HIV reactive for urinary lipoarabinomannan (LAM) measurement. Study staff will obtain and process the samples.

The FIEBRE study will collect these samples for two purposes: for diagnostic tests that are of immediate clinical benefit to patient care, and for research purposes.

This SOP describes the procedures for obtaining samples. Procedures for processing samples are described in SOP F.05, and procedures for testing and storing the samples are described in other SOPs.

5 Supplies and Materials

(See also **APPENDIX to this SOP**: Additional guidance for drawing venous blood from young children)

- Sample logbook [paper or ODK]
- Sample labels with patient's QR code
- Gloves (single-use latex or vinyl)
- Sharps bin
- Butterfly cannula system with connecting tube, or hypodermic needle with syringe (check gauge size)
- Phlebotomy (blood letting) system adaptor (security device)
- 70% alcohol, chlorhexidine-alcohol, chlorhexidine solution, and/or povidone iodine, with cotton wool
- Optional: EMLA cream

- Tourniquet
- Cotton wool, plaster, or gauze with paper tape
- Blood culture bottle (adult or child)
- Mycobacterial blood culture bottle (where appropriate)
- EDTA tube (check volume in tables below)
- Serum tube (check volume in tables below)
- Pharyngeal swabs (plastic handle)
- 10 mL plain Vacutainer or screw-top tube (for pharyngeal swabs)
- Urine container
- Tube rack
- Cooler box [depends on site logistics]

6 Procedures:

6.1 Collecting venous blood

Blood will be collected for testing at the study site, for testing at international reference laboratories, and for future research use. The volume of blood to be collected from a patient depends on the patient's age and body weight, as shown in the table at the end of this SOP.

For additional guidance on drawing blood from small children, see the Appendix at the end of this SOP.

All blood samples must be considered potentially infectious. Study staff must use universal precautions in obtaining and handling blood samples. [Site coordinators: ensure your team has training in universal precautions, infectious risks, have been vaccinated against hepatitis B and that your site has needlestick and PEP procedures]

6.1.1 Arrange all the necessary items on a clean, flat space near the participant, so that you can reach everything easily.

6.1.2 If the participant's skin is visibly dirty over the area where you plan to obtain blood, wash it with soap and water, and allow it to air dry.

6.1.3 Explain to the participant (or to the parent/guardian of a young child) that you are going to draw [a few tablespoons of] blood, as agreed during the informed consent process. Offer to apply EMLA cream if available.

6.1.4 Wash your hands, and put on latex or vinyl gloves.

6.1.5 Remove the cap from the blood culture bottle, clean the rubber septum with 70% alcohol, and allow it to air dry. Be sure the weight of the blood culture bottles is recorded (by you or by the study lab) before adding blood.

6.1.6 Identify the vein you will use (e.g. on the inside of the participant's elbow).

6.1.7 It is very important to clean the skin well before drawing blood for the FIEBRE study, to avoid contaminating the blood cultures:

- Using 70% alcohol, chlorhexidine-alcohol, chlorhexidine solution, and/or povidone iodine, scrub the skin over the vein vigorously for 30 seconds.
- Repeat with fresh swabs or cotton until there is no visible stain on the swab/cotton after wiping.

6.1.8 Allow the skin over the vein to air dry – do not blow on the skin, and do not touch it with your fingers after cleaning.

6.1.9 Following tourniquet application, draw blood into the culture bottle/s and tubes as indicated for the patient's age and weight in the table at the end of this SOP. Always fill the bottles in the order indicated. Be sure to record the weight of the blood culture bottles before and after adding blood.

6.1.10 If a study patient shows signs of severe illness (dehydration, pallor indicating anaemia, low blood pressure), before drawing blood, discuss with the [treating clinician/s and the study site supervisor] to determine the volume to be drawn. In all cases, prioritize the safety and appropriate care of the patient. If the full volume of blood cannot be obtained, adequate volume for tests which will inform patient care (blood cultures, malaria testing) should be prioritized over other study samples. Remove tourniquet once blood has been drawn.

6.1.11 After removing the needle from the participant's arm, immediately place it completely into the sharps bin. Do not recap needles.

6.1.12 Show the patient (or the parent/guardian) how to apply pressure over the vein with cotton wool or gauze to stop the

bleeding and reduce bruising. Cover the site with a plaster, or with gauze/cotton and tape.

6.1.13 **Apply a sample label with the patient's QR code to each tube.**

6.1.14 Gently tilt each tube back and forth 3-4 times and place in the [rack/cooler box.]

6.1.15 During and after the blood drawing, observe the participant for lightheadedness, bleeding or bruising, and manage appropriately.

6.2 Collecting pharyngeal swab samples

Naso- and oropharyngeal (NP and OP) swab samples will be collected to test for respiratory viruses at an international reference laboratory. [OP swabs will only also be collected once local ethics approval has been obtained.] If both OP and NP swabs are obtained for a study participant, both swabs should be received, stored and shipped together in one tube.

6.2.1 Place new, unopened NP/OP swabs (with plastic handles) and new [10 mL plain Vacutainer or screw-top tube] on a clean, flat space near the participant, so that you can reach everything easily. Wear latex or vinyl gloves.

6.2.2 Explain to the participant (or to the parent/guardian of a young child) that you are going to take a swab from the back of her/his nose and the back of his/her mouth. Explain that the procedure may tickle or be uncomfortable, but it should not be painful. If the patient can hold still (with help from the parent/guardian or study staff, if necessary) during the procedure, the collection will be faster and less uncomfortable.

6.2.3 Note: For young children, ask the parent/guardian to hold the child on her/his lap, with the child sitting forward. The parent/guardian should place one arm across the child's body and arms, and the other hand on the child's forehead, to hold the child's head still and facing forward.

6.2.4 Open the [Vacutainer or screw-top tube]. Open the NP swab pack, being careful not to let the cotton tip touch anything between the package and the patient's nostril.

6.2.5 Gently insert the cotton end of the swab into either one of the patient's nostrils until you feel a slight resistance on the swab, indicating that the swab has contacted the back of the nasopharynx. Aim the swab straight back – not up – as in this drawing. (Video at: www.youtube.com/watch?v=DVJNWefmHjE.)

6.2.6 Gently roll the swab for 2-3 seconds to collect the sample and to absorb secretions.

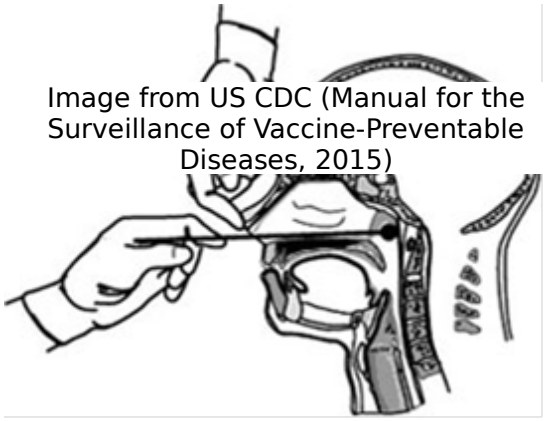

Image from US CDC (Manual for the Surveillance of Vaccine-Preventable Diseases, 2015)

6.2.7 Gently withdraw the swab straight back out of the patient's nostril, and place it directly into the tube. Be careful not to let the swab touch anything between the patient's nostril and the tube.

6.2.8 Cut or snap off the protruding end of the swab

6.2.9 Open the OP swab pack, being careful not to let the cotton tip touch anything between the package and the patient's pharynx.

6.2.10 Insert the OP swab into the patient's mouth and gently roll the swab on both tonsils and the posterior pharynx for 2-3 seconds. Avoid touching the tongue, teeth and gums.

6.2.11 Withdraw the swab and place it directly into the same tube as the NP swab. Be careful not to let the swab touch anything between the patient's mouth and the tube.

6.2.12 Cut or snap off the protruding end of the swab, and replace the lid securely.

6.2.13 **Apply a sample label with the patient's QR code to the tube**, and place the tube in the [rack/cooler box.]

6.3 Collecting a urine sample

A urine sample will be collected from all patients aged ≥2 months and <2 years. A urine sample will be collected from patients aged 2 years and older who are identified by the examining clinician (study staff member) to have: pain when passing urine, frequent urination, tenderness over the urinary bladder, or tenderness over the flank. A urine sample will also be collected from adult patients (>/= 15 years) in Malawi, Mozambique and Zimbabwe who are HIV reactive.

6.3.1 **For babies and small children**

- Prepare a sterile urine cup, a cleansing wipe, and a cold wet (with sterile saline) gauze.
- Wear latex or vinyl gloves.
- Ask the parent/guardian to offer the child a feed (bottle/breast).
- Remove nappy or undergarments.
- (Start timer if using.)
- Gently clean the genital area with the cleansing wipe.
- Apply the cold wet gauze to the child's suprapubic area using a gentle circular motion. This should help to encourage the child to pass urine.
- Wait (or ask the parent/guardian to wait) for the child to urinate spontaneously, and catch the sample into the sterile cup.
- (Stop timer)
- Close the urine container or cup.
- **Apply a sample label with the patient's QR code to the cup**, and place the cup [in a plastic bag then] in the [rack/cooler box.]

6.3.2 For older children and adults

Instruct the patient, or the parent/guardian, on how to collect a "clean catch" urine sample as follows. Give the patient a sterile urine cup and two cleansing wipes.

Instructions for older girls and women:

- Clean hands with soap and water or with alcohol gel as able
- Using the wipes, gently clean around the urethra from front to back, twice.
- Open the cup, being careful not to let anything except urine touch inside the cup or the cap.
- Urinate into the cup. [Try to catch the mid-stream urine if at all possible, i.e. the urine that comes out after the stream begins and before the final drops.]
- Close the cup carefully and bring it back to the study staff.

Instructions for older boys and men:

- Clean hands with soap and water or with alcohol gel as able
- Using the wipes, gently clean around the urethra, twice. If not circumcised, pull back the foreskin before cleaning, and while urinating.

- Open the cup, being careful not to let anything except urine touch inside the cup or the cap.
- Urinate into the cup. [Try to catch the mid-stream urine if at all possible, i.e. the urine that comes out after the stream begins and before the final drops.]
- Close the cup carefully and bring it back to the study staff.

Study staff: Wear latex or vinyl gloves when handling the patient's urine sample. **Apply a sample label with the patient's QR code to the cup**, and place the cup [in a plastic bag then] in the [rack/cooler box.]

7 **Documentation:** FIEBRE protocol (version 2.7, 15 Oct 2018) section 7.3.2, and Sample Log Book [ODK vs paper]. See also Appendix for additional guidance for drawing venous blood from young children, and **Table for blood volumes to be obtained by patient age and weight.**

APPENDIX to SOP F.04: Additional guidance for drawing venous blood from young children
By Shunmay Yeung and Felicity Fitzgerald

Supplies and materials:
24G cannula (for smaller children)
Blunt “drawing up” needle for aspirating blood from cannulas (small children only)
Adhesive clear dressing (eg Tegaderm) for emla/ametop cream)
Emla or ametop cream
Cotton wool

In addition, for children who will be **inpatients :**
0.9% saline drawn into 5 ml syringe
3-way connector
Adhesive clear dressing (eg Tegaderm)
Tape
Splint
Gauze bandage
Cotton wool

- **Patient Preparation**

1. Have patient sit or lie down for the procedure and make them feel comfortable. If patient is a young child, it may be preferable to have the parent/guardian hold the child. If the child is very anxious, ask another team member to help with either distracting or helping the guardian hold the child.

- **Venepuncture procedure for young children**

NOTE: this guideline is for use in younger/smaller children for whom the method of blood culture collection most likely to be successful is via a 24G (yellow) cannula. The phlebotomist must use their clinical judgement as to the exact age cut-off. As a rule of thumb, children >1 year of age could have blood taken via the butterfly method described in the main SOP F.04, but in other circumstances (e.g. plump child with veins that are difficult to see), blood cultures should be taken via a cannula as below even if the child is > 1 year of age. If at any time during the venipuncture procedure the patient displays any adverse reaction (e.g. lightheadedness, fainting, nausea, convulsions) discontinue the procedure immediately and contact a senior clinician for assistance.

1. Remove lids from collection tubes and attach blunt “drawing up” needle to syringe.
2. If the child does not already have a cannula and is going to be admitted to hospital, prepare a flush filling a 5 mL syringe with 0.9% sodium chloride and flush the three-way connector
3. Put on gloves.
4. For sites other than the dorsum of the hand (see 7 below), apply a tourniquet 3-4 inches above the site selected. Ideally, the tourniquet

should not be applied for longer than 1-2 minutes. Leaving it on for an extended period may result in localized stasis and hemoconcentration that can cause erroneous results for some laboratory tests. Beware pinching the skin or compressing an artery.

5. Find a suitable vein. Dorsum of the hand is preferred. The vein running between the 4th and 5th metacarpals is most frequently used. In addition to the usual sites in adults, commonly used sites in children include the volar aspect of the forearm, dorsum of the foot & the great saphenous vein at ankle. Scalp veins should only be used by an experienced phlebotomist. If a suitable vein is not available, contact a member of the ward paediatric team for assistance.
6. Ask the guardian/assistant to stabilise limb by holding joint above & joint below if necessary
7. If using the dorsum of the hand in an infant, grasp as shown in Figure 1: this achieves both immobilisation and tourniquet

Figure 1: holding an infant's hand
(Source: Royal Children's Hospital Melbourne Clinical Practice Guidelines, https://www.rch.org.au/clinicalguide/guideline_index/Intravenous_access_Peripheral/ accessed 21.4.18

8. (Disinfect skin with the site's chosen method, as in the main text of SOP F.04)
9. Allow the disinfectant to dry thoroughly (this is essential for disinfection) Do not re-palpate the vein after disinfecting the site. If you do, repeat the disinfection.
10. Remove the 24G yellow cannula from the package.
11. Insert just distal to and along the line of the vein, angled at 10-15° (Figure 2)

Figure 2: shallow angle of insertion

(Source: Royal Children's Hospital Melbourne Clinical Practice Guidelines, as above)

12. Advance needle and cannula slowly looking for 'flash back'.
13. Once flashback is seen remove the needle and place in the sharps bin, leaving the cannula in place.
14. Aspirate blood for the blood culture using the blunt "drawing up" needle and syringe as per Figure 3.

Figure 3: Aspirating blood for culture.
(Source: Royal Children's Hospital Melbourne Clinical Practice Guidelines, as above)

15. Allow blood to drop passively into collection tubes for other samples as per Figure 4

Figure 4: passive blood collection for infants
(Source: Royal Children's Hospital Melbourne Clinical Practice Guidelines, as above)

16. If the child does not already have a cannula inserted and is going to be admitted to hospital, secure the cannula in place as follows:

- Connect the saline-filled 3-way connector to the end of the cannula by screwing it firmly on. Flush the connector tubing with more saline to confirm intravenous placement.
- In younger children use inverted cross-over straps and another tape over the top (Fig 5)
- Consider placing a small piece of cotton wool ball or gauze underneath the hub of the cannula to prevent pressure areas (Fig 5)
- Place an adhesive clear plastic dressing on top (Fig 6)
- Tapes should secure the limb proximal and distal to the cannula (keeping thumb free) but not too tightly (Fig 7)
- Wrap the whole distal extremity in a gauze bandage.

Fig 5: pad under cannula to prevent pressure areas. IV site should remain visible

Fig 6: secure with tegaderm™ so that IV site is visible

Fig 7: Strap so that joint is immobilised, but avoiding tapes being too tight

(Source: Royal Children's Hospital Melbourne Clinical Practice Guidelines, as above)

17. If blood does not flow, calmly make the following adjustments:
    - Change the position of the needle; pull back if it has penetrated too far, or
      advance the needle if it is not fully inserted in the vein.
    - Make sure the bevel of the needle is up.
    - Loosen the tourniquet which may be on too tight stopping the blood flow.
18. NOTE: If first attempt is unsuccessful repeat the process on another site using a NEW cannula. A clean, sterile cannula must be used for each new collection attempt. If the second attempt fails, do not attempt another. Contact a member of the ward paediatric team for assistance with the venipuncture.
19. Release the tourniquet (if used) as soon as the blood begins to flow. Do not allow the tourniquet to be continuously in place more than 2 minutes.
20. Collect the proper amount of blood for tests, as in main SOP F.04 and Tables.
21. Remove the cannula if child not being admitted and apply pressure to the site with a cotton ball. The guardian may also apply pressure while elevating the arm to assist clotting.
22. Check site to make sure that bleeding has stopped and apply a bandage.

23. Discard cannula in appropriate Sharps container. NEVER RECAP NEEDLES.

24. Discard other materials in appropriate trash receptacle.

25. Before leaving the patient make sure the patient is stable and shows no sign of distress following the procedure.

SOP F-04 and F-05 TABLE: Blood sampling volumes and sequence for patients on Day 0 (version 9.0, 16 Apr 2019) See protocol section 7.3.2.

Filling sequence	ADULTS (≥15 years) AFRICA	ADULTS (≥15 years) ASIA	CHILDREN (<15 years and >7kg)	CHILDREN ≤7kg
1	**Blood culture – 10 mL** (weigh bottle before & after adding blood)		**Blood culture – 4 mL into 4 mL paediatric bottle** (weigh bottle before & after adding blood)	**Blood culture – 2 mL into 4 mL paediatric bottle** (weigh bottle before & after adding blood)
2	**EDTA tube – 2 mL blood** <200 uL whole blood for POCTs and filter paper spots (30 uL malaria micro, 20 uL malaria RDT, 50 uL HIV RDT at African sites, 6 x 10 uL filter paper spots) 250-500 uL whole blood for NAAT ~1300 uL centrifuged → plasma for biomarkers, buffy coat and cell pellet			**EDTA tube – 1.5 mL blood** <200 uL whole blood for POCTs and filter paper spots 500 uL whole blood for NAAT ~800 uL centrifuged → plasma for biomarkers, cell pellet and buffy coat
3	**Plain tube – 9 mL blood** 40 uL for cryptococcal antigen lateral flow assay in all inpatients and all HIV reactive outpatients Centrifuge → 4-5 mL of serum (Discard clot) ≥ 3.1 mL serum for serology ≤ 1-2 mL serum for archive			**Plain tube – volume based on child's body weight:** 2-3 kg 2.5 mL 3-4 kg 4.5 mL 4-5 kg 6.5 mL >5 kg 8.5 mL
4	Mycobacterial cultures (as per SOP F-08b) **5 mL blood**	NONE	NONE	NONE
5	PAXgene for RNA* **2.5 mL blood**	PAXgene for RNA* **2.5 mL blood**	NONE	NONE
Actual minimum blood draw	26 mL (28.5 mL with PAXgene)*	21 mL (23.5 mL with PAXgene)*	15 mL	2-3 kg 6 mL 3-4 kg 8 mL 4-5 kg 10 mL 5-6 kg 12 mL 6-7 kg 14 mL
Maximum blood draw allowed	26 mL (28.5 mL with PAXgene)*	21 mL (23.5 mL with PAXgene)*	15 mL	2-3 kg 6 mL 3-4 kg 8 mL 4-5 kg 10 mL 5-6 kg 12 mL 6-7 kg 14 mL

* PAXgene sample to be obtained only in subset as per central guidance; **ensure local ethics approval**.