| FIEBRE Standard Operating Procedure F.05 | | | |
|---|---|---|---|
| **Title** | **Processing of Patient Samples on Day 0: Blood, Pharyngeal Swabs, and Urine** | | |
| ***SOP Reference*** | ***Version*** | ***Date of effect*** | |
| F.05 | 3.3.5 | 16 Apr 2019 | |

## SOP Development

| | Name | Title | Signature | Date |
|---|---|---|---|---|
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## Review Tracker

| Due date for next review | Reviewer name | Signature | Date reviewed |
|---|---|---|---|
| 31 July 2018 | Kate Haigh | | 20 Nov 18 |
| 18 Dec 2018 | Kate Haigh | | 17 Dec 18 |
| 12 Feb 2019 | Kate Haigh | | 09 Jan 19 |
| 27 Feb 2019 | Kate Haigh | | 13 Feb 19 |
| 27 Apr 2019 | Kate Haigh | | 10 Mar 19 |
| 27 Apr 2019 | Kate Haigh | | 29 Mar 19 |

## 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; correct descriptions of filter paper sampling and storage |
| 3.0 | 20 Nov 2018 (depends on site-specific ethics approval) | Addition of oropharyngeal swab, buffy coat, DBS for D28 and PAXgene samples |
| 3.2.1 | 17 Dec 2018 | Addition of urinary LAM testing (pending confirmation of requirement) on adult samples from sites with HIV prevalence >/= 1% in general adult population. Updating blood volume split between EDTA and plain tube in participants weighing </= 7kg. Possible requirement for cryptococcal antigen testing referenced. |
| 3.3.1 | 09 Jan 2019 | Addition of detail as to how to obtain buffy coat. Confirmation of requirement for urinary LAM and cryptococcal antigen testing. |
| 3.3.2 | 13 Feb 2019 | Clarification that buffy coat should be stored in 0.5ml |

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|---|---|---|
| | | vial |
| 3.3.3 | 10 Mar 2019 | Clear instruction that dried blood spots should be stored at ambient temperature (not refrigerated) |
| 3.3.4 | 29 Mar 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 |
| 3.3.5 | 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

| # | Name (print) | Signature | Date |
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## 1 Title: PROCESSING OF PATIENT SAMPLES ON DAY 0: BLOOD, PHARYNGEAL SWABS, AND URINE

**2 Purpose:** To describe the procedures for processing and storing venous blood, pharyngeal swabs, and urine samples from newly enrolled patients for the FIEBRE study.

**3 Responsible staff:** FIEBRE laboratory staff

**4 Background & Rationale:** On the day of patient enrolment, a venous blood sample and a naso- and oropharyngeal (NP and OP) swab are collected from each patient for study laboratory procedures. In addition, a urine sample is collected from young children, from patients of any age who have symptoms of possible urinary tract infection and [in sites where HIV prevalence >/= 1% in general adult population] all adult inpatients and some adult outpatients [site-specific] who are HIV reactive (for urinary LAM testing). See SOP F.04 for details of how samples are obtained.

After the samples are obtained, they should be brought to the FIEBRE laboratory space for processing as soon as possible. This SOP describes the procedures for preparing the samples for diagnostic tests done at the study site, and for storing the samples for eventual shipping to international reference laboratories.

**5 Supplies and Materials**

- Sample logbook (paper and ODK)
- Samples from one patient: venous blood (in blood culture bottle, EDTA tube, plain tube, +/- mycobacterial blood culture bottle, +/- PAXgene tube), nasopharyngeal and oropharyngeal swab (in sterile tube), +/- urine (in sterile cup)
- Sample labels with patient's QR code
- Gloves (single use latex or vinyl)
- A filter paper disk
- Clean glass slide (for blood smear)
- Malaria RDT test kit
- HIV RDT test kit (for participants ≥15 years and not already known to be HIV reactive, in sites where HIV prevalence is known to be ≥1%)
- Racks for drying filter paper disks

- Small and large plastic bags and dessicant (for storing filter paper samples)
- Tube racks
- Calibrated pipette and pipette tips, or single-use squeezable pipettes
- Centrifuge
- Cryotubes
- Cryotube storage racks/boxes
- Sharps bin
- Biohazard disposal systematic
- Laboratory surface disinfectant (e.g. Virkon)

# 6 Procedures:

## *6.1 Blood culture and mycobacterial blood culture bottles*

Process blood culture bottles as in **SOPs F.08a and F.08b**.

## *6.2 EDTA tube*

The EDTA tube is used to prepare point-of-care tests and filter paper spots, and for whole blood, plasma and buffy coat for storage and future research use.

6.2.1 Ensure the correct patient label is on the EDTA tube, a new filter paper disk, a new malaria RDT, a new glass slide, and a new HIV rapid test (for participants aged ≥15 years in Malawi, Mozambique, and Zimbabwe where test not already completed by government staff).

6.2.2 Wear latex or vinyl gloves. Gently tilt the EDTA tube back and forth a few times to mix the blood in case it has already begun to separate.

6.2.3 Open the EDTA tube, and use the pipette [or blood transfer devices provided with rapid test kits] to place drops of blood on the malaria RDT, glass slide, and HIV rapid test. Follow **SOPs F.06a, F.06b and F.07** to complete the malaria and HIV testing procedures.

6.2.4 Use the pipette to place one (1) blood drop of 10 uL onto each circle of the filter paper disk, to make six (6) blood spots.

6.2.5 Pipette whole blood (volumes as per table at the end of this SOP) into individually labelled cryotubes. Store as per 6.4.3.

6.2.6 Carefully replace the cap of the EDTA tube, and place the tube in a centrifuge bucket.

6.2.7 Place the filter paper disks onto straws/pencils through the central hole, being careful not to touch the filter papers together. Place the filter papers in a clean part of the lab, away from breezes and dust, to dry completely (ideally overnight). When dry, place the filter paper disk in a small plastic sealable bag. Each patient's filter paper disk must be in a separate plastic bag.

6.2.8 Store approximately 50-100 sealed filter papers together in one large sealable plastic bag, along with 20-30 silica desiccant sachets. These filter paper disks should be stored at ambient temperature (not refrigerated)

### *6.3 Plain tube*

The plain tube is used to prepare cryptococcal antigen lateral flow assay (as per SOP F-06d) and serum, which will be stored and shipped to international reference labs for diagnostic testing, and for future research use.

6.3.1 Cryptococcal antigen needs to be tested for from this sample, see SOP F-06d

6.3.2 Ensure the correct label is on the plain tube. Place the tube in a centrifuge bucket.

### *6.4 Centrifuging, aliquoting, and storing blood samples*

6.4.1 Centrifuge the EDTA and plain tube at XXXX rcf or times G force for XX minutes [add correct details for site's centrifuge].

6.4.2 Using a calibrated pipette or sterile Pasteur pipette, aliquot all available sample as plasma, serum, buffy coat for EDTA sample and red cell pellet into individually labelled cryotubes.

For more detail on how to obtain buffy coat; see section 6.5. **See TABLE at the end of this SOP for target volumes for each sample.**

6.4.3 Ensure each cryotube is labelled correctly and place immediately into -80°C freezer. *[Except for PAXgene which should be refrigerated first; see SOP F.20]*

### *6.5 Obtaining buffy coat*

Buffy coat is a layer of white blood cells and platelets, that have been requested by the Rickettsial reference laboratory to improve sensitivity of their serological testing.

6.5.1 After centrifuging the EDTA tube, carefully inspect the tube in order to see the 3 distinct layers; on top, plasma (yellow colour), then a small layer of buffy coat underneath (white colour, classically not much more than 1% of the total sample volume), then at the bottom of the tube the red blood cell layer (red colour)

6.5.2 Push a transfer pipette through the plasma layer to the top of the red blood cell layer, to where the buffy coat is located

6.5.3 Aspirate a small volume, usually between 0.1-0.3ml, which will comprise the buffy coat and a small volume of plasma and red blood cells

6.5.4 Put the buffy coat in a separate 0.5ml cryotube, ensure the tube is labelled correctly and place immediately into a -80°C freezer

6.5.3 Continue with normal procedures regarding obtaining plasma and red cell pellet

### *6.6 Pharyngeal (NP and OP) swabs in sterile tubes*

**[Note:** Both oropharyngeal (OP) and NP swabs may be obtained once ethics approval is in place; check with your study coordinator. If both OP and NP swabs are obtained for a study participant, both swabs should be received, stored and shipped together in one tube.]

6.6.1 Each NP/OP swab should be received, stored and shipped in a dry sterile tube (not in media, and not in a pouch). A plain vacutainer or screw-top tube, sized 2 to 10 mL, is appropriate.

6.6.2 Ensure each NP/OP swab container is labeled correctly, and place as soon as possible into -80°C freezer.

6.6.3 NP/OP swabs should ideally be frozen within 48 hours of being obtained. If a pharyngeal swab cannot be frozen immediately, it may be stored in a refrigerator for ≤7 days. (Don't store swabs at room temperature; refrigeration is necessary to avoid bacterial overgrowth.)

### *6.7 Urine sample*

**To complete urine dipstick testing**

6.7.1 Wear latex or vinyl gloves. Remove a new urine dipstick from the package [and place it on an absorbent pad on the lab benchtop; check package insert, need to avoid contaminating urine in case of need for culture]

6.7.2 Check the patient's label on the urine cup. Open the urine cup, being careful not to let anything touch the inside of the cup or the lid.

6.7.3 [Modify with instructions from urine dipstick package insert: either dip the dipstick into the urine or use a sterile pipette to drop urine onto the dipstick; allow the dipstick to develop for the manufacturer's recommended time period, then read the dipstick]

6.7.4 If the dipstick is positive for nitrite and/or leukocyte esterase, prepare the urine for culture as in SOP F.08c.

6.7.5 If the dipstick is negative for nitrite and leukocyte esterase, replace the lid of the urine cup and dispose of the urine sample with biohazard waste.

6.7.6 Record the urine dipstick result in the patient's CRF [ODK or paper logbook].

**To complete urinary lipoarabinomannan (LAM) testing**

6.7.7 See SOP F-06c for urinary LAM testing and results recording

## 7 Documentation:

- FIEBRE protocol (version 3.0, 31 Oct 2018) sections 7.3.2 and 7.6
- Sample log book (ODK or paper)
- **Table for blood volumes to be obtained by patient age and weight** at the end of this SOP
- See also:

  - SOP F.04 on how to obtain samples

  - SOPs F.06a, F.06b, F.06c, F.06d and F.07 to complete the malaria, HIV, urinary LAM and cryptococcal antigen testing procedures

  - SOPs F.08a and F.08b to process blood culture and mycobacterial blood culture

  - SOP F.08c to perform urine culture

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

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