FIEBRE Standard Operating Procedure F.11			
Title	**Collection and Processing of Patient Samples on Day 28: Blood**		
SOP Reference		***Version***	***Date of effect***
F.11		2.1.3	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		22 Nov 18
20 Dec 2018	Kate Haigh		27 Dec 18
12 Feb 2019	Kate Haigh		16 Apr 19

Revision History

Version No.	Effective date	Reason for change
2.0	22 Nov 2018	Updating to include requirement for EDTA tube, DBS on D28 and blood volume tables
2.1.2	27 Dec 2018	Minor formatting updates
2.1.3	16 Apr 2019	Update to blood volume table appendix to include dried blood spots

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: COLLECTION AND PROCESSING OF PATIENT SAMPLES ON DAY 28: BLOOD

2 Purpose:

To describe the procedures for collecting venous blood from FIEBRE study patients on follow-up Day 28.

3 Responsible staff:

FIEBRE clinical and laboratory staff [adjust based on site personnel]

4 Background & Rationale:

At each patient's Day 28 follow-up visit, venous blood will be taken for serology testing. For best diagnostic results, serology testing requires both "acute" and "convalescent" samples from each patient, taken approximately four weeks apart. Pathogen-specific antibody titers from the two samples are compared to determine the likelihood that a patient's fever was caused by a particular infection.

Obtaining a blood sample on Day 28 is similar to the approach on Day 0, except that on Day 28 we will collect blood for serum (in a plain tube) and EDTA tube. We will not collect blood for culture.

For children who weigh 7 kg or less, again we will use weight-based guidelines to determine the blood volume drawn. For patients with body weight >7 kg and aged <5 years, the maximum amount of blood drawn will be 5 mL. For patients who weigh more than 7 kg, and who are aged ≥ 5 years and <15 years, the maximum amount of blood drawn will be either 5 mL or 10 mL (depending on site ethics and regulatory approvals). For patients aged ≥ 15 years, the maximum amount of blood drawn will be 10 mL.

Samples will be aliquoted, stored and shipped to internationally recognised reference laboratories for serology testing.

This SOP describes the procedures for obtaining, processing and storing the samples.

5 Supplies and Materials

- Sample logbook (paper or Open Data Kit [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)

- Alcohol swabs, or other skin disinfectant
- Optional: EMLA cream
- Tourniquet
- Cotton wool, plaster, or gauze with paper tape
- Serum tube and EDTA tube (check volumes in table below)
- Tube rack
- Cooler box (per site)

6 Procedures:

6.1 Collecting venous blood

Patient blood on Day 28 will be collected 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.

All blood samples must be considered potentially infectious. Study staff must use universal precautions in obtaining and handling blood samples.

6.1.1 Use the same approach as described in SOP F-04 to prepare

6.1.10 **Apply sample labels with the patient's QR code to the tubes**

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

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

6.2 EDTA tube

The EDTA tube is used to prepare filter paper discs and for aliquots for storing and shipping

6.2.1 Ensure the correct patient label is on the EDTA tube and a new filter paper disk

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 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

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

6.2.5 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

6.2.6 Store approximately 50-100 sealed filter papers together in one large sealable plastic bag, along with 20-30 silica desiccant sachets

6.3 Plain tube

The plain tube is used to prepare serum, which will be stored and shipped to international reference labs for diagnostic testing, and for future research use.

6.3.1 Ensure the correct patient 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, whole blood and red cell pellet into individually labelled cryotubes

6.4.3 Ensure each cryotube is labelled correctly and place immediately into -80°C freezer

7 Documentation: FIEBRE protocol (version 3.0, 31 Oct 2018) section 7.3.2 and 7.4.2, and Sample Log Book [ODK vs paper]

NOTE: If your site has ethics approval to draw 10 mL blood for patients with body weight >7 kg and age ≥5 years and <15 years, use Table A.

If your site has approval for only 5 mL for this group, use Table B. See protocol section 7.4.2 for details.

SOP F-11 TABLE A: Blood sampling volumes and sequence for patients on Day 28 (version 3.0, 16 Apr 2019)

Filling sequence	ADULTS (≥15 years)	CHILDREN (≥5 to <15 years, body weight >7 kg)	CHILDREN (<5 years, body weight ≥2 kg)
1	**Plain tube – 9 mL blood** Centrifuge → 4-5 mL of serum (Discard clot)	**Plain tube – 9 mL blood** Centrifuge → 4-5 mL of serum (Discard clot)	**Plain tube – 4 mL blood** Centrifuge → 2-2.5 mL of serum (Discard clot)
2	**EDTA tube: 1 mL blood** 60 uL (6 x 10 uL) filter paper spots ~940 uL centrifuged → plasma for biomarkers, cell pellet		
Actual minimum blood draw	10 mL	10 mL	5 mL
Maximum blood draw allowed	10 mL	10 mL	5 mL

NOTE: If your site has ethics approval to draw 10 mL blood for patients with body weight >7 kg and age ≥5 years and <15 years, use Table A.

If your site has approval for only 5 mL for this group, use Table B. See protocol section 7.4.2 for details.

SOP F-11 TABLE B: Blood sampling volumes and sequence for patients on Day 28 (version 3.0, 16 Apr 2019)

Filling sequence	ADULTS (≥15 years)	CHILDREN (<15 years of any body weight ≥ 2 kg)
1	**Plain tube – 9 mL blood** Centrifuge → 4-5 mL of serum (Discard clot)	**Plain tube – 4 mL blood** Centrifuge → 2-2.5 mL of serum (Discard clot)
2	**EDTA tube: 1 mL blood** 60 uL (6 x 10 uL) filter paper spots ~940 uL centrifuged → plasma for biomarkers, cell pellet	
Actual minimum blood draw	10 mL	5 mL
Maximum blood draw allowed	10 mL	5 mL