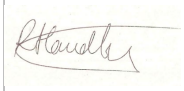
FIEBRE Standard Operating Procedure F20.01			
Title	**Processing and storage of FIEBRE samples at LSHTM**		
SOP Reference		***Version***	***Date of effect***
F-20		1.1.1	06 July 2020

	Name	**Title**	**Signature**	**Date**
Author	Rebecca Handley	LSHTM Laboratory Co-ordinator		02/07/2020
Reviewer	Chrissy h Roberts	LSHTM FIEBRE laboratory expert		04/06/2020
Approver				

Revision History

Version No.	**Effective date**	**Reason for change**

SOP User Confirmation

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

#	**Name (print)**	**Signature**	**Date**
1			
2			
3			

4			
5			
6			
7			
8			
9			
10			

1. **Title**: **Processing and storage of FIEBRE samples at LSHTM**
2. **Purpose:** To describe how to handle and process FIEBRE samples for long term storage and shipping to reference laboratories.
3. **Responsible Staff:** LSHTM FIEBRE lab staff
4. **Background and Rationale:** Specimens collected during the FIEBRE study will be shipped to LSHTM. A number of these specimens will require splitting before being shipped onwards to international and national reference laboratories for further diagnosis. The remaining specimens will be split into lower volume aliquots for long term storage.
5. **Supplies and Materials**
   - 5.1. FIEBRE samples
   - 5.2. -80 freezer
   - 5.3. FluidX Impression whole rack scanner
   - 5.4. FluidX Scope Individual barcode scanner
   - 5.5. Extra-long nitryl gloves
   - 5.6. Blue roll
   - 5.7. Ziplock bags
   - 5.8. 70% Ethanol
   - 5.9. Biocleanse
   - 5.10. DNA-Exitus Plus spray
   - 5.11. Pipettes and pipette tips
   - 5.12. FIEBRE tube organiser
   - 5.13. Laptop with biobanking software installed
   - 5.14. Tablet with ODK installed
   - 5.15. FluidX barcoded tubes (0.5ml and 1ml)
   - 5.16. FluidX 96-well barcoded racks for sample storage

6. Procedures

6.1. Reference lab allocation

6.1.1. For some participants there may be a limited volume of serum to send a specimen to all the required reference labs

6.1.2. Ref lab allocation will be determined prior to any sample processing in the lab

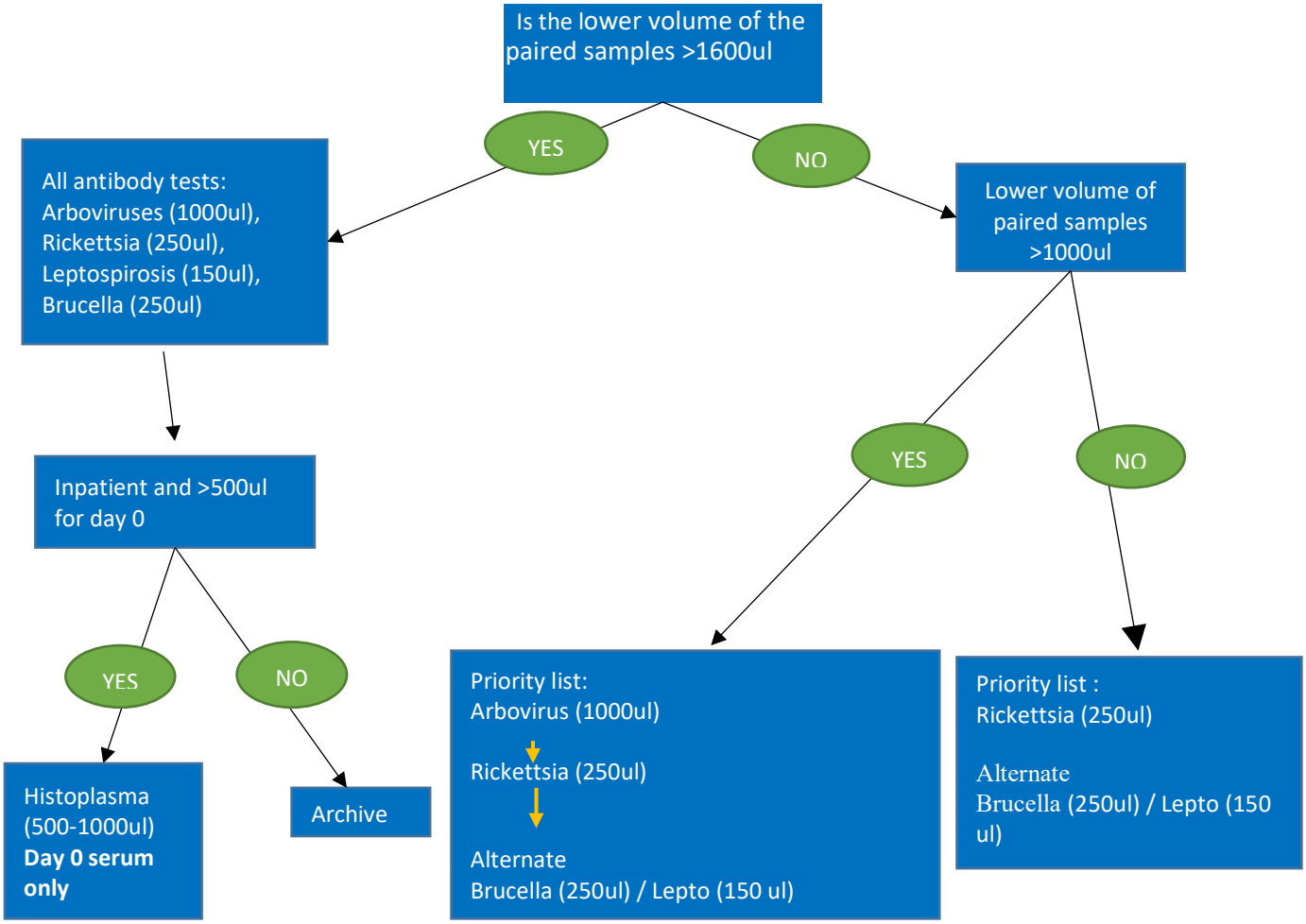
6.1.3. For low-volume samples a flow chart will be used to determine what reference labs to allocate samples to (figure 1).

6.1.4. True or false for each laboratory should be entered into a spreadsheet alongside the sample ID for each participant in a batch of samples.

6.1.5. Prior to aliquoting this spreadsheet should be uploaded onto the FIEBRE ODK server.

6.1.6. This can be over-ruled when aliquoting.

Figure 1: Flow chart of reference lab allocation



6.2. Aliquoting serum specimens for reference labs and long-term storage

6.2.1. The FIEBRE specific COP must be followed when handling all FIEBRE samples

6.2.2. All samples will be handled within a Class I cabinet within the 425a. Secondary containment must be used when storing samples within the -70 °C freezer and when handled outside of the cabinet. This will mean storing individual cryoboxes/FluidX racks within sealable plastic bags and decontaminating with ethanol for at least a minute prior to removing from the cabinet.

6.2.3. Thaw specimens to be aliquoted within the Class I cabinet

6.2.4. Once thawed, place all serum samples from one participant in the FIEBRE tube holder (Figure 2).

6.2.5. Scan barcodes of serum sample and use the ODK form to determine which laboratories to allocate samples. Place the corresponding FluidX 0.5ml and 1 ml tubes in the designated slots within the tube holder.

6.2.6. For day 0 samples if you have excess specimen prioritise sending to additional reference labs rather than archiving. Record this on the ODK form.

6.2.7. Carefully pipette the required volume of serum into the necessary tubes and seal each tube. If there is sample remaining pipette 50 – 100ul into a 500 ul tube for Leishmania and Cryptococcus and split the remaining serum for the archive (4 x 50ul and the remaining in one or two tubes).

6.2.8. Scan the barcodes of the filled tubes when prompted by the ODK form

6.2.9. Move samples to each labelled FluidX rack, there will be a rack for each reference laboratory, one for Cryptococcus and Leishmania aliquot and one rack for archive samples.

6.2.10. Disinfect surfaces and gloves with 70% ethanol and DNA Exitus-plus after each sample.

6.2.11. At the end of each day transfer aliquots into their permanent racks and store n the -70 freezer. Once a permanent rack is full use the whole-rack scanner to record the location of each tube within the new rack.

6.2.12. Place in the freezer and record this position on the LIMS system.

Figure 2: FIEBRE tube organiser

Pathogen	Volume
Arboviruses	1ml
Rickettsia	250 ul
Brucella	250 ul
Leptospirosis	100 ul
Histoplasma	0.5ml-1ml
Leishmania/CrAg	50-100ul

Table 1: Serum volumes required for each reference lab

6.2 Aliquoting plasma

6.2.1 Plasma should be aliquoted into smaller volumes for leptospirosis testing and long term storage in the repository.

6.2.2 Like serum, all plasma samples processing should take place in the Class I cabinet. Samples should be removed from the freezer and allowed to thaw in the Class I MSC

6.2.3 500ul of plasma is needed for leptospirosis testing. Aliquot this into a 1ml FluidX tube and seal using the purple screw caps. Record the barcode into the ODK software.

6.2.4 Split the remaining serum into a maximum of five 50ul samples and one larger volume aliquot.

6.2.5 Record aliquot volume, plasma lab ID and the new tube ID in the ODK software and store in a separate FluidX rack to the reference lab samples.

6.2.6 Once a box is filled use the whole rack scanner to record the position of each tube and the rack barcode.

6.2.7 Place in the freezer and record this position on the LIMS system

6.2.8 Complete this with the remaining samples.

END