FIEBRE Standard Operating Procedure F.08b			
Title	**Blood Mycobacterial Culture Preparation, Interpretation, and Results Recording**		
SOP Reference		***Version***	***Date of effect***
F.08b		1.2.1	18 Dec 2018

SOP Development

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Due date for next review	Reviewer name	Signature	Date reviewed
31 July 2018	Kate Haigh		21 Nov 18
20 Dec 2018	Kate Haigh		18 Dec 18
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Revision History

Version No.	Effective date	Reason for change
1.2.1	18 Dec 2018	Updating to reflect requirement for mycobacterial blood cultures to be taken only from adult HIV reactive participants in HIV prevalance >/= 1% sites (inpatient only or inpatient and outpatient depending on site)

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: BLOOD MYCOBACTERIAL CULTURE PREPARATION, INTERPRETATION, AND RESULTS RECORDING

2 Purpose: To describe the procedures for processing, interpreting and reporting FIEBRE study blood mycobacterial culture results using Myco/F Lytic bottles.

3 Responsible staff: FIEBRE microbiology laboratory staff

4 Background & Rationale: Mycobacterial bloodstream infections are a rare, but serious, cause of fever. HIV-positive people are more at risk of mycobacterial infections. Mycobacterial blood cultures will be performed for HIV reactive FIEBRE *inpatients* aged 15 years and older at sites where HIV prevalence is >1% in the general adult population (Malawi, Mozambique, Zimbabwe). In the Malawi site only, mycobacterial blood cultures will also be performed for HIV reactive FIEBRE *outpatients* aged 15 years and older.

This SOP describes general procedures for processing and storing mycobacterial blood culture samples obtained as part of the FIEBRE study. For specific details on individual steps, please refer to your laboratory's accepted SOPs, manuals and procedures. Feel free to ask the FIEBRE study co-ordinator and your laboratory supervisor for guidance whenever needed.

Bactec Myco/F Lytic bottles are optimised to allow growth of mycobacteria and fungi. Since most study sites do not have access to a Bactec machine, these bottles will be incubated manually. The bottles contain a sensor at the bottom that changes fluorescence with increasing CO_2 concentration. Changes in the fluorescence can be seen with a Woods Lamp in a dark room or cabinet. The bottles do not contain any antibiotics, so they also will grow other bacteria from the blood and contaminants.

5 Supplies and Materials

- Sample logbook (paper or ODK)
- Blood culture logbook or records (paper or ODK)
- Participant's blood sample in mycobacterial blood culture bottle
- Sample labels with patient's QR or other barcode
- Gloves (single-use latex or vinyl)

- **For a complete list of materials and reagents needed for each Procedure mentioned in this FIEBRE study SOP, refer to your lab's local SOPs**
- Laboratory weighing scales
- Sharps bin
- Biohazard disposal system

6 Procedures:

Always wear latex or vinyl gloves when handling blood culture specimens, to prevent exposure to blood borne pathogens. Observe standard universal precautions when obtaining and handling blood cultures.

6.1 Weighing mycobacterial blood culture bottles before use

Weigh each mycobacterial blood culture bottle, to a precision of 0.1g or better, before issue to the clinical team. Record the weight [on the bottle's label/in ODK/paper log – per site].

6.2 After a patient's blood is added to a mycobacterial blood culture bottle:

6.2.1 Keep mycobacterial blood culture bottles in an air-conditioned room (or cool box or refrigerator) if the ambient temperature is >23° C. Storing a blood culture bottle in warm or hot conditions for hours risks damaging the growth of micro-organisms.

6.2.2 Ensure that mycobacterial blood culture bottles are delivered to the microbiology lab on the same day that patient blood is added.

6.3 Receiving mycobacterial blood cultures in the microbiology laboratory:

The following notes are general guidance for processing FIEBRE study blood cultures. Refer to your lab's SOPs for specific details. **Myco/F Lytic bottles should only be sampled in a Containment Level 3 facility.**

6.3.1 Before opening the transport bag, check that the bottle inside has not been damaged in transit. If the bottle is cracked or leaking, do not open the transport bag. Throw the unopened bag and bottle into biohazard waste bin (and record this result).

6.3.2 Check to ensure that the study ID on the bottle and associated paperwork match.

6.3.3 Register receipt of the culture in the lab register or LIMS system [per site].

6.3.4 Weigh the bottle, to a precision of 0.1g or better, and record the filled weight [on the bottle's label/in ODK/paper log – per site. (The weight of the cap will be accounted for separately.)

6.3.5 Place the bottle in the incubator.

6.4 Incubation for mycobacterial blood cultures

6.4.1 Examine bottles on day 3, 4 or 5 (to avoid weekend work), then weekly for 6 (six) weeks. Remove each bottle from the incubator and examine it in a dark room or hood using a Wood's lamp (UV lamp); compare fluorescence with a positive control bottle.

6.4.2 Return negative bottles (no fluorescence) for further incubation. Bottles that are positive (show fluorescence) should be handled in a BSL-2 cabinet in a Containment Level 3 facility.

6.4.3 If a bottle is positive, use a sterile syringe to remove a small volume (approximately 0.5 to 2 mL) of culture supernatant.

6.4.4 Fix and inactivate two or more slides inside the cabinet before removing for Gram and Ziehl-Neelsen (acid-fast) staining.

6.4.5 If mycobacteria are seen, then proceed to **grow and identify according to local SOPs**. Cryopreserve the isolate in duplicate as described below and/or according to **local SOPs**.

6.4.6 If non-mycobacteria are seen on staining and no mycobacteria are present, then send the bottle to a Containment Level 2 laboratory for further identification. Continue isolate identification and cryopreservation unless the same organism was isolated in the blood culture bottle.

6.4.7 Any yeasts seen on Gram stain may be Containment Level 3 organisms (e.g. *Histoplasma capsulatum*). Continue any further processing only within the CL 3 facility. Identify the organism to the limit of the capability of the lab and cryopreserve the isolate as described below and/or according to **local SOPs**.

6.4.8 If no positive signal is seen by the end of 6 weeks of incubation, record the result as "negative" and dispose of the bottle according to local protocols.

6.5 Cryopreserving and archiving isolates

6.5.1 **For FIEBRE, we will cryopreserve all micro-organisms isolated from mycobacterial blood cultures.**

6.5.2 To prepare a positive mycobacterial blood culture isolate for storage, sub-culture the isolate on solid media (e.g. Loewenstein-Jensen, Stonebrink, or 7H10). Incubate as usual until a heavy mycobacterial growth is achieved (several weeks for *M. tuberculosis*).

6.5.3 Suspend mycobacteria either in NaCL with 5% glycerol or in liquid mycobacterial media (for example Mgit, Kirchner, or Middlebrook). The bacterial suspension should be highly concentrated (>2 McFarland).

6.5.4 Add 1 mL of the bacterial suspension to each of two 2 mL cryovials. If bacteria are suspended in NaCL with glycerol, freeze and store the aliquots as below.

6.5.5 If bacteria are suspended in liquid media, culture the cryovials at 37° C for an additional 4 (four) weeks to allow further bacterial growth. Then freeze and store the aliquots as below.

6.5.6 Label each of the duplicate cryovials with both sample and patient identifiers. Use at least two patient identifiers [standardize labelling per site].

6.5.7 Place one cryovial in a cryobox to be sent to the reference lab, and store the duplicate vial at your study site.

6.5.8 Freeze the vials at -70° C or below.

6.5.9 [Record in the freezer log book/culture logbook; per site.]

6.6 Reporting mycobacterial culture results

Report all microbiology results to the patient's clinical team in real time, using appropriate local procedures [per site].

7 Documentation:

- FIEBRE protocol (version 2.5, 31 Jul 2018) section 7.6.4
- Local laboratory SOPs for use of incubator, Gram and acid-fast stain, identification and susceptibility testing of mycobacteria/microorganisms, cryopreservation of microorganisms, Containment Level 3 and Biosafety Level 2 cabinet procedures, reporting of mycobacterial culture results to clinical teams, and other routine microbiology laboratory procedures