FIEBRE Standard Operating Procedure F.08a		
Title	**Blood Culture Preparation, Interpretation, and Results Recording**	
SOP Reference	***Version***	***Date of effect***
F.08a	1.1.2	18 Dec 2018

SOP Development

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Due date for next review	Reviewer name	Signature	Date reviewed
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Revision History

Version No.	Effective date	Reason for change
1.1.2	18 Dec 2018	Minor formatting updates

SOP User Confirmation

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

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1 Title: BLOOD CULTURE PREPARATION, INTERPRETATION, AND RESULTS RECORDING

2 Purpose: To describe the procedures for processing, interpreting and reporting FIEBRE study blood culture results using the automated blood culture analyser.

3 Responsible staff: FIEBRE microbiology laboratory staff

4 Background & Rationale: Bacterial and fungal bloodstream infections are a rare, but serious, cause of fever. Detection of these infections is an important part of good clinical care, and a key element of the FIEBRE study.

This SOP describes general procedures for processing and storing 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.

Some FIEBRE sites use the **BacT/Alert 3D** blood culture system. This system uses a colorimetric sensor and reflected light to determine the amount of carbon dioxide (CO_2) that is dissolved in the culture medium. If micro-organisms are present in a blood sample, CO_2 is produced as the organisms metabolize the substrates in the culture medium. When growth of the micro-organisms produces CO_2, the colour of the sensor in the bottom of each culture bottle changes from green to yellow.

A light-emitting diode (LED) projects light on the sensor. The light reflected is measured by a photodetector. As more CO_2 is generated, more light is reflected. This information is transmitted to a computer where it is compared to the initial CO_2 level in the bottle. If there has been a sustained acceleration in the rate of CO_2 production, high initial CO_2 content, and/or an unusually high rate of CO_2 production, the sample is determined to be positive.

Some FIEBRE sites use the **Becton Dickinson Bactec** machine. These machines work on a similar principle to BacT/Alert; the difference is that with Bactec the sensor changes fluorescence rather than colour.

For the purposes of FIEBRE SOPs, we will refer to both the BacT/Alert system and the Bactec system as **automated blood culture (ABC) machines**.

For culture-positive samples from FIEBRE patients, cryopreserved isolates will be shipped on dry ice to an international reference laboratory for confirmation of identification and of antimicrobial susceptibility.

5 Supplies and Materials

- Sample logbooks or records (paper or Open Data Kit [ODK])
- Blood culture logbooks or records (paper or ODK)
- Participant's blood sample in blood culture bottle
- Sample labels with patient's QR code
- Gloves (single-use latex or vinyl)
- Laboratory weighing scales
- **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**
- 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 blood culture bottles before use

Weigh each 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 – site-specific].

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

6.2.1 Keep 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 blood culture bottles are delivered to the microbiology lab within 12 hours of the patient's blood being added.

6.3 Receiving 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.

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].

6.3.5 Check the transport time of the bottle since blood was added. If the time between phlebotomy and incubation is >12 hours, then check that the sensor on the bottom of the bottle has not changed colour, from grey to yellow (BacT/Alert) or started to develop fluorescence (Bactec). This will require experience and judgment; ask senior microbiology lab staff for assistance if needed. If the sensor has changed colour or fluorescence, or if there is any doubt, sample the bottle as described below. Then place the bottle in the ABC machine as usual.

6.3.6 Scan the bottle into the machine ABC [entering the study number in the patient information section – site specific logging method.] Put the bottle into an available slot in the ABC machine to begin incubation. **Refer to your lab's manual or SOP for specific details on machine operation.**

6.4 Incubation in the automated blood culture (ABC) machine

6.4.1 Standard incubation temperature is 37° C (acceptable range 36-37° C). Standard duration of incubation is 5 days.

6.4.2 Check the ABC machine for any positives results at least twice per day, ideally in the morning and afternoon.

6.5 When the ABC machine signals that a bottle is positive:

6.5.1 Standard incubation temperature is 37° C (acceptable range 36-37° C). Standard duration of incubation is 5 days.

6.5.2 Remove the bottle as soon as possible. Clean the bottle top with an alcohol swab, and let it dry.

6.5.3 Sample the bottle using either a sterile syringe or a sterile venting unit, placing a small volume (0.5 to 2 millilitres; more is fine, but not necessary) of culture broth into a sterile receptacle (e.g. bijoux bottle), or directly onto plates or slides [per site].

6.5.4 Perform a Gram stain; **refer to your site's SOP for Gram stain**.

6.5.5 If no organisms are seen on Gram stain, set up a single chocolate agar plate with the culture broth and incubate in CO_2 (either CO_2 incubator or candle jar; refer to **your site's SOP** for this procedure). Return the bottle to the machine.

6.5.6 If organisms are seen on Gram stain, proceed to **identification and susceptibility testing according to your site's SOPs**.

6.5.7 Report all positive microbiology results to the clinical team and study coordinator as soon as possible [per site]. Record the results in [ODK/culture logbook].

6.5.8 The next day – or as soon as pure cultures are obtained on non-selective agar (e.g. chocolate, blood, etc) – cryopreserve some colonies as described in section 6.7 below.

6.5.9 Identify all isolates to at least genus level, where possible. Record the best estimate of species name in all cases. (This is required for specimen shipping and import to the FIEBRE microbiology reference laboratory.)

6.5.10 Perform antibiotic susceptibility testing by standard guidelines; **refer to your lab's SOPs**.

6.5.11 Report the results of the identification and sensitivity tests to the clinical team and study co-ordinator.

6.5.12 Retain the culture bottle until all identification and susceptibility testing procedures are complete, and the isolate has been saved.

6.6 After 5 (five) days of incubation with no growth, the culture is negative.

6.6.1 Take the bottle out of the machine.

6.6.2 Record the negative result in [ODK/culture logbook].

6.6.3 Dispose of the bottle according to your lab's standard procedures [e.g. autoclave and discard in biohazard waste].

6.7 Cryopreserving and archiving isolates

6.7.1 **For FIEBRE, we will cryopreserve <u>all</u> micro-organisms isolated from blood cultures.**

6.7.2 Use a culture that is pure, on a non-selective medium such as blood agar, and less than 24 hours old.

6.7.3 For each isolate, prepare <u>two</u> 2 mL sterile cryovials containing 1 mL each of 10-20% glycerol in Brain Heart Infusion, Tryptone-Soy broth, or similar. Freeze either with glycerol as a preservative, or use commercially prepared cryobeads.

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

6.7.5 Select several typical colonies and make a thick suspension in the medium. If using cryobeads, gently mix the beads and liquid by inverting the cryovial (with its cap on), then pipette away excess liquid. (Discard the liquid waste according to manufacturers' instructions and your local lab SOPs.)

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

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

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

6.8 Containment Level 3 pathogens

If a Containment Level 3 organism is suspected at any stage in the identification process, refer to local SOPs and standards.

All CL3 pathogens should be stored separately from non-CL3 pathogens.

6.9 Reporting microbiology results

Report all microbiology results to the clinical team and study coordinator in real time, using appropriate local procedures [per site].

7 Documentation:

- FIEBRE protocol (version 2.5, 31 Jul 2018) section 7.6.4
- “Effects of delayed-entry conditions on the recovery and detection of microorganisms from BacT/ALERT and BACTEC blood culture bottles,” Sautter, et al, *J Clin Microbiol.* 2006 Apr;44(4):1245-9
- Local laboratory SOPs for use of automated blood culture machine, Gram stain, identification and susceptibility testing of microorganisms, cryopreservation of microorganisms, management of Containment Level 3 organisms, reporting of microbiology results to clinical teams, and other routine microbiology laboratory procedures