FIEBRE Standard Operating Procedure F.06d		
Title	**Cryptococcal antigen testing and results recording**	
SOP Reference	***Version***	***Date of effect***
F.06d	1.1.3	2 Jan 2019

SOP Development

	Name	Title	Signature	Date
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Review Tracker

Due date for next review	Reviewer name	Signature	Date reviewed
27 Feb 2019	Kate Haigh		29 Jan 19
29 Mar 2019			

Revision History

Version No.	Effective date	Reason for change
1.1.2	21 Jan 2019	Addition of information about storage of tests
1.1.3	29 Jan 2019	Clarification re freezing of samples in event of test delay

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: CRYPTOCOCCAL ANTIGEN TESTING AND RESULTS RECORDING

2 Purpose: To describe the procedures for testing and recording results of tests for cryptococcal antigen for participants in the FIEBRE study.

3 Responsible staff: FIEBRE laboratory staff [adjust based on site personnel]

4 Background & Rationale: Cryptococcal meningitis causes approximately 15% of AIDS-related mortality annually (Park et al, 2009). The highest burden is in sub-Saharan Africa, where cryptococcal meningitis is cited as the most common form of adult meningitis (Rajasingham et al, 2015).

Cryptococcal antigen (CrAg) testing will be performed on a venous sample on all inpatients recruited to FIEBRE and on HIV reactive outpatients. Cryptococcosis is much more common in immunosuppressed individuals. Therefore, all HIV-reactive participants will have CrAg testing done, and all inpatients, as they will be the most unwell cohort and thus more likely to be immunosuppressed.

5 Supplies and Materials

- Sample logbook (paper or ODK)
- Cryptococcal antigen testing results form (paper or ODK)
- IMMY CrAg lateral flow assay testing kit: includes LF specimen diluent (3.0ml), LF titration diluent (6.0ml), CrAg LF test strips, CrAg positive control (1ml)
- Participant's blood sample in plain (serum) tube
- Sample labels with patient’s QR code
- Gloves (single-use latex or vinyl)
- Tube rack
- Calibrated pipette or pipette tips (40microlitre and 80microlitre)
- Timer
- Disposable micro-centrifuge tubes, test tubes or micro-titre plate
- Sharps bin
- Biohazard disposal system

6 Procedures:

6.1 Collecting serum blood sample

6.1.1 Collect samples as per SOP F.04

6.2 Storage of CrAg Lateral Flow Assay materials

6.2.1 All reagents included in this kit should be stored at 20-25ºC until use or the expiration date listed on the reagent labels

6.2.2 Unused test strips should be stored in the LF test vial with the dessicant cap firmly attached

6.3 Quality assurance of CrAg Lateral Flow Assay

Quality assurance (QA) of CrAg testing strips should be completed for every packet (50 test strips). If you have already done QA for the current batch, proceed to section 6.3

6.3.1 A positive control can be evaluated by adding 1 drop of LF specimen diluent followed by 1 drop of CrAg positive control to a tube, then proceed to 6.2.3

6.3.2 A negative control can be evaluated by adding 2 drops of LF specimen diluent to a tube

6.3.3 Insert a test strip into the tubes and read after 10 minutes as per section 6.4

6.3.4 With each packet of testing strips that passes QA, write 'QA passed' on the packet

6.3.5 If the result is not as expected, document and return to manufacturer. If there is QA or test failure, document the outcome and freeze the sample for testing at a later date

6.4 Preparing CrAg Lateral Flow Assay

6.4.1 If a delay is encountered in specimen processing, storage at 2-8ºC for up to 72 hours is permissible. Serum/plasma can be stored for longer periods at <-20ºC, provided they are not repeatedly thawed and refrozen

6.4.2 Apply 40microlitres (or 1 drop) of LF specimen diluent to an appropriate reservoir (disposable micro-centrifuge tube, test tubes etc)

6.4.3 Add 40microlitres of specimen to the reservoir and mix

6.4.4 Submerge the white end of a CrAg lateral flow test strip into the specimen

6.4.5 Wait 10 minutes and read the result

6.5 Reading CrAg Lateral Flow Assay

6.5.1 The presence of two lines ('test' and 'control'), regardless of the intensity of the test line, indicates a positive result

6.5.2 A single 'control' line indicates a negative result

6.5.3 If the control line does not appear, the results are invalid and the test should be repeated

6.6 Recording and reporting the CrAg test result

6.6.1 Record the CrAg test result in the patient's ODK record or paper logbook

6.6.2 Report the result as soon as possible to the clinical team managing the study participant

7 Documentation:

- FIEBRE protocol (version 3.0, 31 Oct 2018) sections 7.3.2 and 7.6.8
- SOP F.04