FIEBRE Standard Operating Procedure F.06c		
Title	**Processing urinary lipoarabinomannan (LAM)**	
SOP Reference	***Version***	***Date of effect***
F.06c	1.2.2	10 Mar 2019

SOP Development

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Due date for next review	Reviewer name	Signature	Date reviewed

Revision History

Version No.	Effective date	Reason for change
1.1.2	16 Jan 2019	Included requirement for batch positive control testing
1.1.3	21 Jan 2019	Documentation updates re control testing
1.1.4	29 Jan 2019	Clarification re documentation of QC passing of lots
1.2.1	01 Mar 2019	Clear instruction regarding necessity of double reading of strips and addition of clear 'indeterminate' category
1.2.2	10 Mar 2019	Clarification that each read should be independent

SOP User Confirmation

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

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1 Title: PROCESSING URINARY LIPOARABINOMANNAN

2 Purpose: To describe the procedures for processing urine samples to test for lipoarabinomannan (LAM) for participants in the FIEBRE study.

3 Responsible staff: FIEBRE laboratory staff

4 Background & Rationale: The urinary LAM antigen test detects whether LAM antigen, a lipopolysaccharide present in mycobacterial cell walls, which is released from metabolically active or degenerating bacterial cells, is detected in the urine.

HIV reactive individuals are more at risk of mycobacterial infection, and as such, at study sites where HIV prevalence is >/= to 1% in the general population, it is expected that a proportion of febrile illness will be due to mycobacterial infection.

Urinary LAM testing has been recognised by the World Health Organisation as demonstrating improved sensitivity in seriously ill HIV reactive individuals, especially those with low CD4 counts.

In sites where HIV prevalence is >/= 1% FIEBRE study will test for urinary LAM in HIV reactive participants deemed to be seriously unwell; this will incorporate all HIV reactive inpatients at these sites and each site will individually decide whether to do this test on HIV reactive outpatients.

On enrolment into the study, a urine sample should be obtained and brought to the FIEBRE laboratory space for processing as soon as possible.

5 Supplies and Materials

- Sample logbook (paper and ODK)
- Alere Determine TB LAM test kit
- Positive control solution
- Quality assurance log (example in Appendix 1)
- Calibrated pipette and pipette tips, or single-use squeezable pipettes

- Sample labels with patient's QR code
- Gloves (single-use latex or vinyl)
- Biohazard disposal system
- Lab surface disinfectant (e.g. Virkon)
- Sharps bin
- Timer

6 Procedures:

6.1 Collecting urine sample

Collect urine sample as described in SOP F.04.

6.2 Storage of urine sample

6.2.1 Fresh urine samples can be used within 8 hours if kept at room temperature, however, in order to generate the most reliable results the test should be completed as soon as possible

6.2.2 Urine samples should be stored at 2-8ºC if the test is delayed and to be run within 3 days of collection. If testing is delayed more than 3 days, the samples should be frozen (-20ºC or cooler)

6.2.3 For frozen or refrigerated samples bring to room temperature one hour before use

6.2.4 Frozen samples may contain aggregates; all thawed samples must be centrifuged at 10,000g for 5 minutes at room temperature and the 60microlitre test sample should be carefully collected from the clear supernatant. Avoid repeated freeze/thaw cycles. Specimens that have been frozen and thawed more than 3 times cannot be used

6.3 Quality assurance of urinary LAM test kits

If you have already quality assured the lot you are using, proceed to section 6.4.

6.3.1 For each new lot of Alere Determine TB LAM Ag test kits received, and for each new delivery, quality assurance testing with positive control solution must be undertaken. For each lot, document the

number on the lot card in the quality assurance log (example in Appendix 1)

6.3.2 Wear latex or vinyl gloves. Remove one test unit from the card strip by bending and tearing at the perforation. Removal of the test units should start from the right side of the test card to preserve the lot number which appears on the left side of the test card. The control testing should be initiated within 2 hours after removing the protective foil cover from each test

6.3.3 Remove the protective foil cover from the test

6.3.4 Apply 60µL of positive control solution (or 2 drops) to the sample pad (white pad marked by the arrow symbol)

6.3.5 Wait a minimum of 25 minutes and two people should read the result. Visualise the strip under standard indoor lighting conditions or in the shade. Do not visualize the strip under direct sunlight. Results are stable for up to 35 minutes after sample application. Do not read beyond 35 minutes

6.3.6 The test should read positive i.e. purple/grey bars appear in both the control window (labelled “Control”) and the patient window (labelled “Patient”) of the strip. The test result is positive even if the patient bar appears lighter or darker than the control bar

6.3.7 If the test reads positive, document that the batch has been quality assured, write 'QA passed' on all packets in the lot and proceed to testing patient samples

6.3.8 If the test reads anything other than positive (see section 6.4.8), document on quality assurance log and return to manufacturer

6.4 Processing urine sample

6.4.1 Wear latex or vinyl gloves. The desired number of test units from the 10-test card can be removed by bending and tearing at the perforation. Removal of the test units should start from the right side of the test card to preserve the lot number which appears on the left side of the test card. Initiate the assay within 2 hours after removing the protective foil cover from each test

6.4.2 Check the patient’s label on the urine cup. Open the urine cup, being careful not to let anything touch the inside of the cup or the lid

6.4.3 Remove the protective foil cover from each test

6.4.4 Ensure each test is labelled with correct participant ID

6.4.5 Apply 60µL of sample (or 2 drops of urine) to the sample pad (white pad marked by the arrow symbol)

6.4.6 Results must be read by 2 people. If the 2 readers interpret the test differently, a third person must be available to make the decision. Each read should be independent, without knowing the opinion of the other readers

6.4.7 Wait a minimum of 25 minutes and read result. Visualise the strip under standard indoor lighting conditions or in the shade. Do not visualize the strip under direct sunlight. Results are stable for up to 35 minutes after sample application. Do not read beyond 35 minutes (see Figure 1)

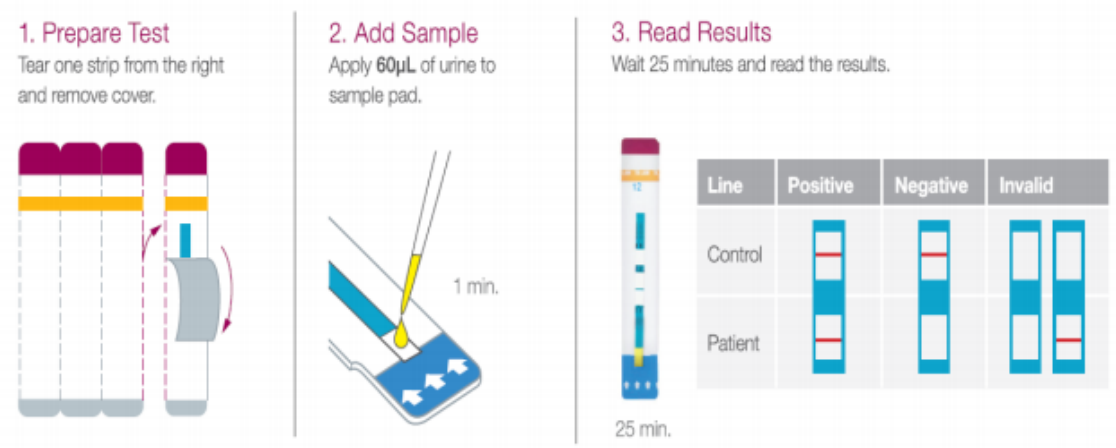

Figure 1: Urinary LAM testing procedure

6.4.8 Read the test result; to assist with results reading and interpretation, use the reference scale card (provided in the kit) by holding it alongside the patient window. Possible results are:

- POSITIVE: purple/grey bars appear in both the control window (labelled “Control”) and the patient window (labelled “Patient”) of the strip. The test result is positive even if the patient bar appears lighter or darker than the control bar

- NEGATIVE: one purple/grey bar appears in the control window of the strip (labelled “Control”) and no purple/grey bar appears in the patient window of the strip (labelled “Patient”)

- INVALID: if there is no purple/grey bar in the control window of the strip, even if a purple/grey bar appears in the patient window of the strip, the result is invalid and the test should be repeated

- INDETERMINATE: one purple/grey bar appears in the control window of the strip (labelled “Control”) with unclear or incomplete purple/grey bar in the patient window of the strip (labelled “Patient”)

6.4.9 As above, if the 2 readers reach different conclusions (for example one thinks the test is positive and one invalid), a third person must be available to make the definitive conclusion

6.4.10 Record the result in the patient's CRF (ODK or logbook)

6.4.11 Where there is a positive result, inform the clinical team looking after the participant to guide patient management

7 Documentation:

- FIEBRE protocol (version 3.0, 31 Oct 2018) section 7.3.2
- Sample log book (ODK or paper)
- See also SOP F.04 on how to obtain samples
- Alere Determine TB LAM Ag package insert
- Quality assurance log

APPENDIX 1

Quality assurance log for Alere Determine TB LAM Ag test kits

Date of QA test (DD/MM/YY)	Lot number	Tested by?	Which control solution used?	Test reads positive (Y/N)	If no, date returned to manufacturer? (Y/N)