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|  | <b>Section: <i>Tuberculosis (TB)</i></b>             |                     |
|   | <b>Title : TB LAM Ag test [Alere] [SSDTTUBT100T]</b> |                     |
|   | <b>Number:</b>                                       | <b>Version:</b>     |
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**The interpretation of the result must always be made using the reference scale card included in each kit (check interpretation part).**

### 1.0. Purpose

The purpose of this standard operating procedure (SOP) is to detail the steps for correctly performing, interpreting, and documenting valid results for TB LAM Ag Lateral Flow Assay (LFA). TB LAM Ag LFA is an immunoassay for the detection of lipoarabinomannan (LAM) antigen of Mycobacteria in human urine as an aid in the diagnosis of active mycobacterial infection in HIV positive individuals with clinical symptoms of tuberculosis.

### 2.0. Scope

The procedure applies to all facilities performing urine TB LAM Ag Lateral flow Assay to assist in the diagnosis of TB in HIV infected adults with signs and symptoms of TB (pulmonary or extra pulmonary) who have a low CD4 count (CD4 < 200 cells/ul according to local protocol) or who are seriously ill (WHO stage III/IV).


### 3.0. Responsibility and Authorization

The persons responsible for performing this test are Laboratory Technologist and trained non-laboratory personnel (e.g Nurses, HTC counselors).

### 4.0. Materials

- 4.1. TB LAM Lateral Flow Assay kit/card
  - 4.1.1. TB LAM Ag test Strips
  - 4.1.2. TB LAM Positive Control (1mL)
- 4.2. Materials required but not provided in the kit
  - 4.2.1. Timer
  - 4.2.2. Gloves
  - 4.2.3. Pipettor 60µL and 60µL tips (these can be replaced by disposable pipettes of similar volumes)
  - 4.2.4. Sharps discard containers
  - 4.2.5. Pen and sharp permanent marker
  - 4.2.6. Biohazard disposable bags

### 5.0. Safety, Health & Environment

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Treat all urine specimens as potentially infectious and follow basic universal precautions. Wear protective clothing (coat/apron and gloves) when handling the specimens.

## 6.0. Principle

Alere Determine TB LAM Ag is an immunochromatographic test for the qualitative detection of lipoarabinomannan (LAM) antigen of Mycobacteria in human urine. Alere Determine TB LAM Ag employs highly purified antibodies specific to the major polysaccharide antigen of the genus Mycobacterium: lipoarabinomannan (LAM). These antibodies are used for both the capture and the detection tracer. The capture antibodies are adsorbed onto the nitrocellulose membrane of the test strip. The detection antibody is labelled by conjugation to colloidal gold particles.

After a urine specimen is added to the sample pad, the colloidal gold conjugated antibodies attach to the LAM antigen and are released by the specimen from the conjugate pad. This immunological complex is then captured by anti LAM antibodies immobilized on the nitrocellulose membrane and made visible due to the presence of the colloidal gold label. A positive result (a visible purple/gray line) indicates that LAM antigen of Mycobacteria is present in the sample at or above the detection limit of the test; whereas a negative result (no visible purple/gray line) indicates it is not present or below detection limit. To ensure assay validity, a procedural control bar is incorporated in the assay device.


## 7. Specimen collection and storage

Collect midstream urine in a fresh standard urine collection container. Fresh urine samples can be used within 8 hours if kept at room temperature. Morning urine is the best sample.

- Urine samples should be stored at 2-8°C if the test is to be run within 3 days of collection.
- If testing is delayed more than 3 days, the samples should be frozen (-20°C or colder). For frozen or refrigerated urine bring all samples to room temperature one hour prior to use. Frozen samples may contain aggregates.
- All thawed samples must be centrifuged at 10,000 g for 5 minutes at room temperature and the 60 µL 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.

## 8.1 Reagent storage and preparation

Alere Determine TB LAM Ag test cards must be stored at 2-30°C until the expiration date. Kit components are stable until expiration date when handled and stored as directed. Do not use kit

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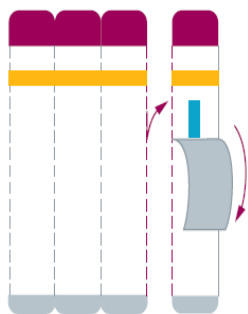
components beyond expiration date. Immediately reseal all unused tests in the foil pouch containing the desiccant by pressing seal from end to end to close. Do not use devices that have become wet or if the packaging has become damaged.

## 8.2 Test Procedure

- 8.2.1 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. The assay should be initiated within 2 hours after removing the protective foil cover from each test.
- 8.2.2 Remove the protective foil cover from each test. Label the cryovial tube identical with EDTA tube containing the patient blood to be analyzed. See figure 1.
- 8.2.3 Apply 60 µL of sample (or 2 drops of urine) to the sample pad (White pad marked by the arrow symbol).
- 8.2.4 Result should be interpreted between 25 and 35 minutes after sample application. Visualize the strip under standard indoor lighting conditions or in the shade. Do not visualize the strip under direct sun light. Do not read beyond 35 minutes.

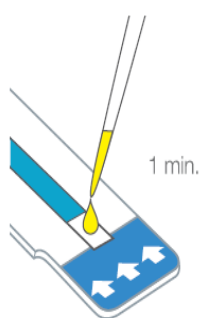
### 1. Prepare Test

Tear one strip from the right and remove cover.



### 2. Add Sample

Apply 60 µL of urine to sample pad.



### 3. Read Results

Wait 25 minutes and read the results.



25 min.








| Line    | Positive  | Negative  | Invalid   |
|---------|---|---|---|
| Control |  |  |  |
| Patient |  |  |  |

Figure 1 TB LAM Ag testing procedure

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### 9.0 Interpretation of test results

Warning: the interpretation of the result must be made using the reference scale card (provided in the kit) by holding it alongside the patient window in a well-lit environment. The test is pos ONLY if **the “Patient” line is equal to or stronger than any of the colored lines in the “POSITIVE” range** on the Reference Scale Card (see Figure 2). Faint line (lighter than grade 1) should be considered as indefinite/equivocal (check section).

In case of any doubt test must be done again on a new fresh sample (morning urine is ideal).

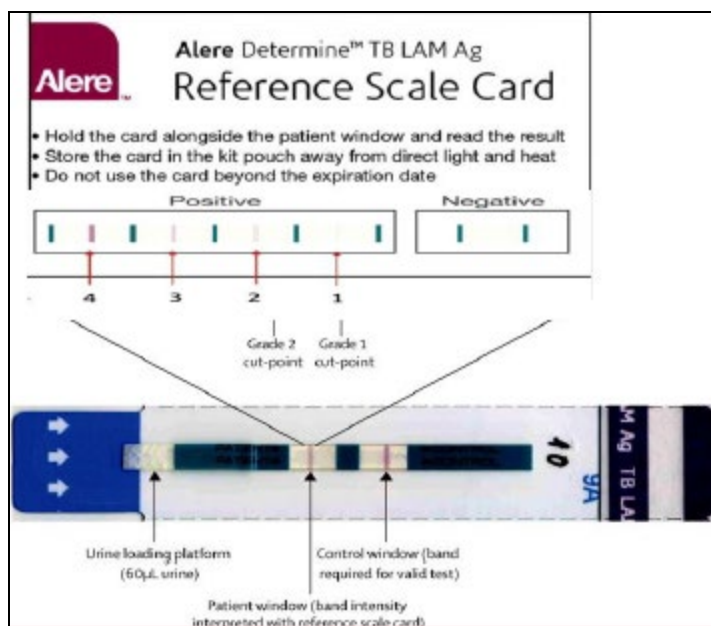



Figure 2: Interpretation of urine TB LAM Ag LFA results

#### LAM Antigen POSITIVE (Two Bars - Control and Patient Bars)

The test result is positive even if the patient line appears lighter or darker than the control line, **as long as the “Patient” line is equal to or stronger than any of the colored lines in the “POSITIVE” range** on the Reference Scale Card

#### NEGATIVE (One Bar)

One purple/gray bar appears in the control window of the strip (labelled “Control”) and no purple/gray bar appears in the Patient window of the strip (labelled “Patient”).

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### INVALID (No Bar)

If there is no purple/gray bar in the control window of the strip, even if a purple/gray bar appears in the patient window of the strip, the result is invalid and the test should be repeated. If the problem persists, contact your local distributor or call Alere Technical Support as detailed below.

### EQUIVOCAL/INDEFINITE:

One purple/gray bar appears in the control window of the strip (labelled “Control”) with unclear or incomplete purple/gray bar in the patient window of the strip (labelled “Patient”). Faint line (lighter than grade 1) must not be considered as positive.

For a better clinical decision the test should be repeated on a fresh sample. Early morning urine is best.

## 10.0 Quality control testing (**NOT PART OF THE ALERE KIT**)

Leftover urine samples with TB-LAM 4+ positive result, on the Alere TB-LAM test, can be aliquoted into small cryotubes and frozen (-20°C) for use as control samples. Thaw one of the frozen aliquots and conduct Quality control for TB LAM test weekly. In the case where there are no specimens to be test for TB LAM, Quality Control may not be Evaluated in that week. Record quality control results in the TB LAM result log book.


### 8.3.1 Follow the following procedure to evaluate TB LAM Ag quality control:

#### TB LAM Ag positive control

- First label the test strip with TB LAM Positive control
- Add 60ul of the TB LAM Ag positive control on the labeled test strip.
- Read results after 25 minutes

#### TB LAM Ag Negative control

- Label the test strip, TB LAM negative control
- Add 60ul of saline/distilled water
- Read results after 25 minutes

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## 11.0 Limitations

- Alere Determine™ TB LAM Ag is designed to detect Mycobacterial LAM antigen in human urine. Other samples (e.g. sputum, serum, plasma, CSF or other body fluids) or pooled urine specimens should not be used.
- No test provides absolute assurance that a specimen does not contain low levels of LAM antigen such as those present at a very early stage of infection. A negative result does not preclude the possibility of infection with *M. tuberculosis*.
- Testing diuretic urine may affect the ability of the test to detect LAM antigen in urine of TB patients.
- Excretion of LAM antigen in urine may vary depending on the individual patient's condition and his underlying illness or treatment. The effect of treatment of the patient with broad-spectrum antibiotics on the Alere Determine™ TB LAM Ag test performance has not been established
- Alere Determine™ TB LAM Ag does not differentiate between the various species of Mycobacterium, such as *M. tuberculosis*, *M. leprae*, and *M. avium*. In an area endemic for tuberculosis the LAM antigen detected in a clinical sample is likely to be attributed to *M. tuberculosis*.
- No test is 100% specific. Positive results should be evaluated in light of the overall clinical evaluation before a diagnosis is made.
- Alere Determine™ TB LAM Ag should be followed up with confirmation test such as bacterial culture and with a drug susceptibility test, if possible.
- The intensity of the patient bar does not necessarily correlate to the bacterial burden.

## 10.0 REFERENCES

Alere Determine LAM Ag Lateral Flow Assay package insert