# TB LAM Ag Lateral Flow Assay
## Standard Operating Procedure

### 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 LFA is 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 CD4<100cells/ul 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 bud 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

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 labeled 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.

## 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.

- 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 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 2drops of urine) to the sample pad (White pad marked by the arrow symbol).

8.2.4 Wait a minimum of 25 minutes and read result. Visualize the strip under standard indoor lighting conditions or in the shade. Do not visualize the strip under direct sun light. Results are stable for up to 35 minutes after sample application. Do not read beyond 35 minutes.

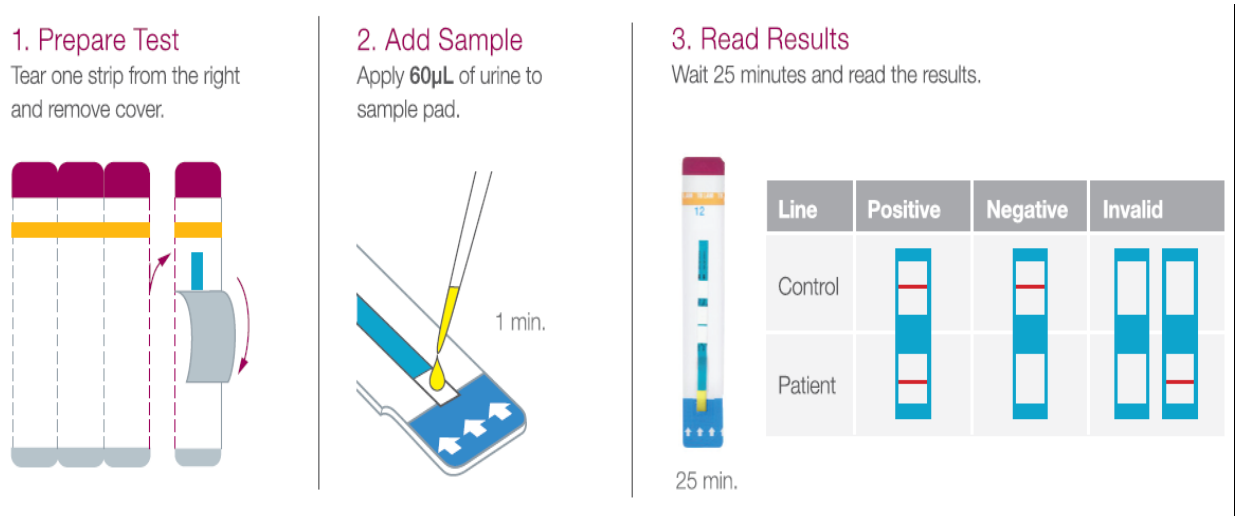


Figure 1 TB LAM Ag testing procedure

## 9.0 Interpretation of test results

To assist with results reading and interpretation, use the reference scale card (provided in the kit) by holding it alongside the patient window.

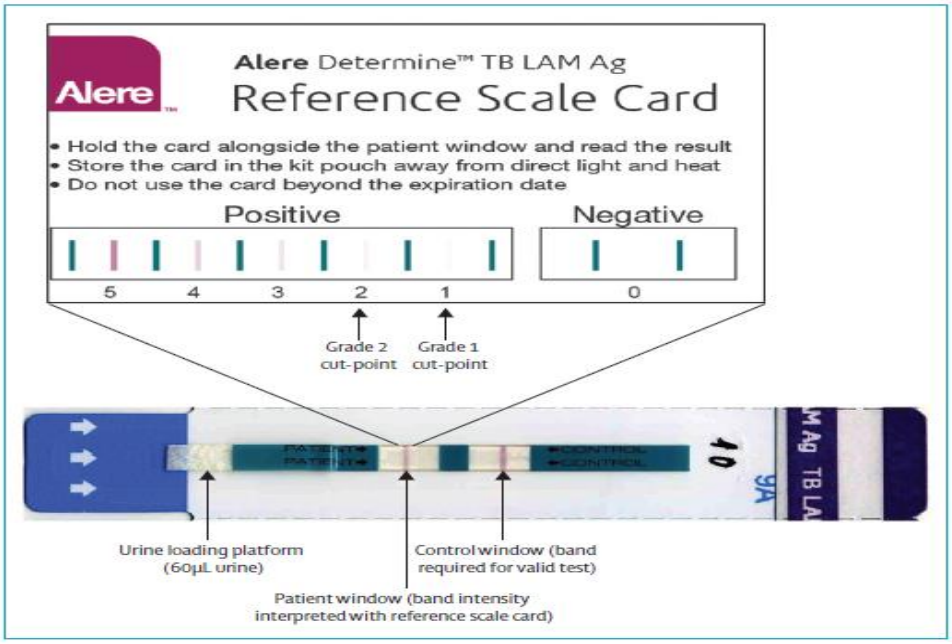


Figure 2: Interpretation of urine TB LAM Ag LFA results

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

Purple/gray bars appear in both the control window (labelled "Control") and the Patient window (Labelled "Patient") of the strip. Note: The test result is positive even if the patient bar appears lighter or darker than the control bar.

**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").

**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.

**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"). For a better clinical decision the test should be repeated. Alternatively, collect a new urine sample in the following days from the patient and test. Early morning urine is recommended.

## 8.3 Quality control testing

Conduct Quality control for TB LAM test weekly, before you test the first specimen to be analyzed during that particular week. 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 1 drop 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 2 drops of saline/distilled water
- Read results after 10 minutes

## 9 REFERENCES

Alere Determine LAM Ag Lateral Flow Assay package insert