A CLINICAL REFERENCE GUIDE FOR HEALTHCARE PROVIDERS IN SOUTH AFRICA

GUIDANCE DOCUMENT

Guidance on the use of the Lateral flow urine lipoarabinomannan assay for the diagnosis of active tuberculosis in people living with HIV

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CONTENTS

List of abbreviations and acronyms

Ag	Antigen
AHD	Advanced HIV disease
AlereLAM	Determine™ LF-LAM Ag
ART	Antiretroviral therapy
BMI	Body mass index
CD4	Cluster of differentiation 4
CI	Confidence interval
CrI	Credible interval
CSF	Cerebrospinal fluid
DST	Drug susceptibility testing
GXP	GeneXpert
HIV	Human immunodeficiency virus
LAM	Lipoarabinomannan
LF-LAM	Lateral flow lipoarabinomannan
LPA	Line probe assay
MTB	Mycobacterium tuberculosis
NIDS	National Indicator Data Set
NIMART	Nurse-initiated management of antiretroviral therapy
NIMDR-TB	Nurse-initiated management of drug-resistant tuberculosis
NTP	National Tuberculosis Programme
PLHIV	People living with HIV
POC	Point-of-care test
Pre-XDR TB	Pre-extensively drug-resistant tuberculosis
QC	Quality control
RIF	Rifampicin
RTC	Regional training centres
SOP	Standard operating procedure
TB	Tuberculosis
USA	United States of America
WHO	World Health Organization
XDR-TB	Extensively drug-resistant tuberculosis
Xpert	GeneXpert

Key definitions

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Advanced HIV disease (AHD) is defined as a CD4 cell count of fewer than 200 cells/μL or a WHO clinical Stage 3 or 4 at presentation for care. All children with HIV who are aged under 5 years should be considered as having AHD at presentation. However, it should be noted, that children under 5 years who are stable on ART should not be classified as having AHD.

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Seriously ill patients (adult norms) are defined based on four danger signs: respiratory rate of more than 30 breaths per minute, a temperature of more than 39 °C, heart rate of more than 120 beats per minute, unable to walk unaided and/or have a low BMI (<18.5) and/or severely underweight or wasted.

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For children, signs of serious illness include lethargy or unconsciousness; convulsions; unable to drink or breastfeed; and repeated vomiting. Other clinical conditions such as body temperature ≥39 °C and age-defined tachycardia and/or tachypnoea can be considered based on clinical judgement (WHO 2017, Guidelines for managing AHD and rapid initiation of ART).

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Age groups: the following definitions for adults, adolescents and children are used in these guidelines:

1. an adult is a person older than 18 years of age
2. an adolescent is a person 10–18 years of age inclusive
3. a child is a person under 10 years of age

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Inpatient health care setting is a health care facility where patients are admitted and assigned a bed while undergoing diagnosis and receiving treatment and care, for at least one overnight stay. These facilities may include the following settings: Day hospitals, medical wards, emergency wards and community health centres.

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Outpatient health care setting is a health care facility where patients are undergoing diagnosis and receiving treatment and care but are not admitted for an overnight stay, e.g. clinic, community health care centres, outpatient departments.

1. Purpose of this Document

This document guides health care providers on the use of the lateral flow lipoarabinomannan assay (LF-LAM) for the diagnosis and management of active tuberculosis in people living with HIV (PLHIV).

2. Background

Tuberculosis (TB) is the leading cause of death in HIV-positive individuals,[1] accounting for nearly half a million cases and 32% of AIDS-related deaths in 2017.[2] The strategy of the World Health Organization (WHO) for TB prevention, care and control for 2015–2035 (known as the End of TB Strategy) prioritises the early diagnosis of TB as a critical strategy for reducing the global burden of TB.[3] Those with suppressed immunity due to HIV infection are significantly more likely to develop active TB than those with normal immunity. An estimated 50–60% of HIV-positive people infected with TB will go on to develop the active disease.[4] TB becomes more difficult to diagnose as immunosuppression progresses. Immunosuppression is also associated with disseminated disease and high mortality risk in PLHIV.

Underdiagnosis of TB, especially in places without widespread health care access, is a principal barrier to combating the disease. While newer, improved molecular diagnostics such as the Xpert MTB/RIF® Ultra assay have good sensitivity and excellent specificity and are widely available in South Africa, the turnaround time for results remains a critical issue in the country.

The diagnosis of TB in PLHIV using sputum-based tests is also challenging, given the frequency of extrapulmonary TB and paucibacillary pulmonary TB, and the inability of such patients to expectorate sputum. As a result, TB/HIV-infected clients often have increased mortality due to rapid TB disease progression, late diagnosis and late treatment initiation. Newer tests based on the detection of the mycobacterial lipoarabinomannan antigen in urine have proven to be efficacious as point-of-care (POC) tests for TB among people with AHD.

It should be noted that the currently available urine LAM assays have suboptimal sensitivity and are therefore not suitable as diagnostic tests for TB in all populations. However, unlike traditional diagnostic methods, urine LAM assays demonstrate greater sensitivity for the diagnosis of TB among individuals coinfected with HIV compared to HIV seronegative individuals.

LF-LAM WILL SERVE AS A USEFUL TOOL TO FACILITATE EARLY DIAGNOSIS AND EARLY TREATMENT INITIATION IN HIV-POSITIVE PATIENTS AND THEREBY REDUCE MORTALITY.

WHY NOW? CONTEXTUALISING THIS GUIDANCE DOCUMENT

In 2019, the WHO Global TB Programme convened a Guideline Development Group to provide updated clinical guidance and management on the use of the LF-LAM assay for the diagnosis of TB in PLHIV. These revised guidelines have expanded eligibility for use in patients with CD4 counts to equal or less than 200 cells/µL (versus the previous recommendation of a CD4 count of fewer than 100 cells/µL). Also, the approach to managing both inpatients and ambulatory patients has been updated. Since then, the 2019 Cochrane review on the use of LF-LAM in PLHIV has also strengthened available evidence.[5]

However, the WHO guidelines do not speak to the integration of LF-LAM and GeneXpert (as well as other tests) in broader diagnostic algorithms and protocols. Local studies have shown that for sputum-expectorating hospitalised patients with AHD, where access to both tests is available, concurrent testing with Xpert and LAM is likely the best strategy for diagnosing TB. Further, urine LAM testing in sputum-scarce hospitalised HIV-infected patients has also been shown to effectively diagnose TB.[6,7]

Because the evidence base is limited for the outpatient setting for patients with AHD and/or low CD4 counts, clear direction is required at this stage. Therefore, this guidance document serves to clarify South Africa's use of the lateral flow urine lipoarabinomannan assay in the context of diagnosing active TB in PLHIV in the country, using the most recent evidence-based global recommendations.

3. Rationale

The emergence of easier to use POC tests using urine to identify TB (by detecting the mycobacterial LAM antigen), has been a welcome addition to the TB diagnostics environment. LF-LAM (Determine™ TB LAM Ag; Abbott, Chicago, IL, USA) can be used for the diagnosis of TB in HIV-positive patients with advanced immunodeficiency. The Global Plan to Stop TB has prioritised the development of simple, accurate, inexpensive tests for TB case detection in HIV-positive individuals.[4] As a strategy for rapid TB diagnosis, the detection of Mycobacterium tuberculosis antigens has been explored over several decades.[8] Lipoarabinomannan (LAM), a 17.5 kD glycolipid component of the outer cell wall of mycobacteria, is an attractive diagnostic target for several reasons. LAM as a bacterial product has the theoretical potential to discriminate active TB from latent TB infection independent of human immune responses. It is heat-stable, cleared by the kidney and detectable in urine.[9] A urine test could facilitate TB diagnosis in patients in whom sputum is uninformative or not obtainable and lacks the infection control risks associated with sputum production or blood collection.

Since 2015, several studies on the use of the LF-LAM assay have been conducted. New evidence has emerged that potentially justifies the use of the test in a broader group of patients. Evidence emerging from the plethora of studies has noted the increased sensitivity of the test among patients with a low CD4 count ≤200. It is not clear why this is the case, but it could be due to the high bacillary and antigen load and/or increased glomerular permeability resulting in high antigen levels in urine or TB disease affecting the genitourinary tract considering that disseminated/EPTB is common in this group of patients. LF-LAM has several characteristics that make it well suited for use for the South African health context, thereby providing a strong justification for the national roll-out to all levels of care. Finally, urine LAM detection may be amenable to simple, inexpensive POC platforms. Some of the evidence-based recommendations are outlined below.

Greater sensitivity and improved yield in patients with AHD

The sensitivity of the assay depends on the level of immunosuppression (higher at lower CD4 counts), and it has been reported to be around 50% in patients with CD4 below 200 cells/μL.[10] A systematic review found a pooled sensitivity of 42% (95% credible interval (CrI) 31–55), increasing to 54% (CrI 38–69) among people with CD4 ≤100 cells/μL.[11]

This recommendation was informed by the Broger et al. (2019) systematic review and meta-analysis of 12 cross-sectional or cohort studies that showed a relatively low pooled sensitivity of 45% (95% CI 29–63) and high specificity of 92% (80–97) against a microbiological reference standard. In a subgroup of patients with HIV and CD4 counts less than or equal to 100 cells/μL, pooled sensitivity was 56% (41–70).[12]

Strong evidence for reducing morbidity and mortality in children with AHD

Prospective paediatric cohort studies found a pooled sensitivity of 47% (95% CI 27–69) and specificity of 82% (95% CI 71–89) (WHO).[13]

Lower specificity in children could refer to cross-reactivity of Determine™ LAM Ag with bacteria from perineal skin or stool that contaminate the urine sample during collection, as urine bags may remain on the skin for several hours until the child produces urine.[14]

Reduced mortality in people living with HIV

The LF-LAM assay also serves to identify patients at a higher risk of mortality. Two clinical trials performed in South Africa have shown a reduction in mortality among hospitalised patients who immediately initiated treatment after a positive LF-LAM result. There is now good evidence to support mortality reductions with the use of LF-LAM in HIV-positive patients admitted to hospital.[14,15]

Urine LAM testing can be useful for TB diagnosis in HIV-positive TB-symptomatic patients with no CD4 cell count. The LAM grade can identify patients at higher risk of death in this situation.[16]

Bedside LAM-guided treatment initiation in HIV-positive hospital inpatients with suspected TB has been associated with reduced mortality.[17]

Although the STAMP trial has shown that urine-based TB screening did not reduce overall mortality in all HIV-positive inpatients, the urine-based TB screening may contribute to mortality reductions in some high-risk subgroups and implementation could contribute towards global targets to reduce TB mortality. [15]

Point-of-care diagnosis improves early case detection in risk populations

A POC test that readily detects active TB has been shown to reduce diagnostic delays, interrupt transmission through appropriate treatment and address many of the current gaps in global TB control.[18]

Rapid turnaround time and ease of use, to support early treatment initiation

The test provides results in 25 minutes and it is easy to perform.[4]

Cost-effective in low-income settings

Studies have shown that including LAM in the TB diagnostic algorithms is cost-effective.[6]

Currently, the LF-LAM assay is the only commercially available TB POC test that has been recommended by the WHO for the diagnosis of TB in HIV-positive adults with symptoms of TB who are severely immunocompromised and/or seriously ill.[5] Several hypotheses may explain the higher sensitivity of urine LAM detection in PLHIV.

SUMMARY TABLE OF MAJOR GUIDELINE CHANGES

This guidance document provides a summary of changes adopted from recommendations by the WHO policy update (2019), on the LF-LAM assay for the diagnosis of active tuberculosis in people living with HIV.

WHO STRONGLY RECOMMENDS USING LF-LAM TO ASSIST IN THE DIAGNOSIS OF ACTIVE TB IN HIV-POSITIVE ADULTS, ADOLESCENTS AND CHILDREN

CLINICAL SETTING	PREVIOUS SOUTH AFRICAN GUIDANCE	WHO RECOMMENDATIONS	CURRENT RECOMMENDATIONS FOR THE SOUTH AFRICAN CONTEXT
Inpatient Setting	LF-LAM can be administered for seriously ill patients, with advanced HIV in hospitalised settings when they are seen for a medical diagnosis in the emergency room or are admitted to medical wards irrespective of whether TB is suspected or not or the patient's CD4+ count.	Irrespective of signs and symptoms of TB (pulmonary and/or extrapulmonary) and with a CD4 cell count of fewer than 200 cells/µL • With AHD Stage 4 or who are seriously ill, irrespective of CD4 count.	The guidance adopts recommendations to include the use of LF-LAM to assist in the diagnosis of active TB in HIV-positive patients irrespective of whether TB is suspected or not (i.e. irrespective of signs and symptoms of TB) and irrespective of the patient's CD4+ count, and irrespective of whether AHD is present or not. A sputum molecular test for TB (e.g. Gene-Xpert) should be performed in parallel. See Algorithm chart on page 17.
Outpatient Setting	For outpatients (ambulatory patients seen in community health care centres, primary health care settings day hospitals, including ART initiation clinics), LF-LAM should only be performed when: • TB is suspected based on symptoms and/or signs AND • CD4 count ≤100 cells.	• With signs and symptoms of TB (pulmonary and/or extrapulmonary) or seriously ill • Irrespective of signs and symptoms of TB and with a CD4 cell count of fewer than 100 cells/mm.^{3}	The guidance adopts recommendations to include the use of LF-LAM to assist in the diagnosis of active TB in HIV- positive patients with: • Signs and symptoms of TB (pulmonary and/or extrapulmonary) and • CD4 count < 200 cells/mm^3 or AHD Stage 4 or who are seriously ill.

In addition to recommendations provided by the WHO, this guidance further expands and elaborates on the use of LF-LAM eligibility criteria for hospitalised patients with HIV, irrespective of presumptive TB or availability of CD4 count test results based on the following assumptions:

- If a patient is HIV-positive and is admitted to hospital, they are likely to have advanced HIV and/or be severely unwell.
- The diagnosis of advanced HIV in adults would be based on CD4 count or staging.

THIS POLICY GUIDANCE ALSO REINFORCES THE NEED TO PERFORM GENEXPERT TESTING TO EXCLUDE RIFAMPICIN RESISTANCE.

4. About the Test

This is a qualitative lateral flow test used to detect the lipoarabinomannan antigen of Mycobacteria in human urine. This antigen is a lipopolysaccharide found in the outer cell wall of the Mycobacterium. Lipoarabinomannan antigens are released by metabolically active or degenerating Mycobacterium cells and are detectable in urine following clearance by the kidneys. It is therefore indicative of active TB.

The sensitivity of the test is higher in patients with a low CD4 count ≤200. It is not clear why this is the case but could be due to the high bacillary and antigen load, increased glomerular permeability resulting in high antigen levels in urine or TB disease affecting the genitourinary tract, considering that disseminated/EPTB is common in this group of patients.

The test is performed manually by applying 60 µL of urine to the Determine™ TB LAM Ag test strip at room temperature for 25 minutes. The strip is evaluated visually. The intensity of any visible band on the test strip is graded by comparing it with the intensities of the bands on a manufacturer-supplied reference card. The reference card includes four bands (ranging from Grade 1 representing a very low-intensity band to Grade 4 representing a dark/high-intensity band).

5. Criteria for the Use of this Test

The urine-based LF-LAM assay is a commercially available POC test for the detection of active TB. This is an immunocapture assay that detects LAM antigen in urine. LF-LAM assay is used to assist in the diagnosis of TB in PLHIV who are seriously ill and hospitalised or in HIV-coinfected clients in outpatient settings with advanced HIV disease and/or CD4 ≤200 cells/µL.

In South Africa, the LF-LAM assay test may be performed as a POC test, which can be administered in the consulting room as long as:

1. The test is consistently stored at the right temperature.
2. The test user adheres to the waiting time before determining a result.
3. The result is always determined using the reference card.
4. The result is recorded and captured in the correct documentation.

5.1 BENEFITS OF THE TEST

Urine-based testing has advantages over sputum-based testing because urine is easy to collect. In addition, some of the benefits of the test include the following:

- The test is easy to perform; minimal training is required.
- The test can be done at a POC facility and can assist with the diagnosis of active TB; however, it should be noted that the exact location or strain of the TB cannot be determined.
- The test will ensure early identification and early initiation of treatment and reduction in mortality for HIV/TB-coinfected patients.

5.2 LIMITATIONS OF THE TEST

- LF-LAM Ag should be followed up with a test to confirm Rifampicin sensitivity such as Xpert if possible, but this should not delay treatment initiation for patients with positive LF-LAM results.
- The intensity of the patient's result bar does not necessarily correlate to the bacterial burden.
- Excretion of LAM antigen in urine may vary depending on the individual patient's condition and their underlying illness or treatment. For example, the use of diuretics may affect the ability of the test to detect the LAM antigen in urine. This should be considered when interpreting the results by reviewing the patient's medical records or obtaining a medical history from the patient.

- Invalid results are not a sign of a positive or negative test result. If the result is invalid, the test should be repeated. If repeated invalid results are obtained, the patient should be re-tested by another method. The final patient diagnosis should be assessed based on a comprehensive clinical evaluation.
- The test does not distinguish between the different species of mycobacteria, and therefore will need to be used in combination with confirmatory tests such as GeneXpert MTB/RIF® Ultra assay, or culture and LPA/DST.
- The test may only be used with a urine sample.

5.3 INPATIENTS

The LF-LAM Ag assay can be administered to all hospitalised patients with an HIV infection that warrants clinical suspicion of TB. In these patients, LF-LAM and Xpert should be used to assist in the diagnosis of TB disease regardless of the presence of TB symptoms or clinical danger signs and availability of CD4 count results.

- The tests should not be used if the packaging is damaged or test strips are wet.
- The foil pouch with remaining tests together with the desiccant must be resealed immediately after removal of a test strip, by pressing the seal from end to end to close.

Seriously ill patients are more likely to test positive with the LF-LAM test than ambulatory patients. Seriously ill patients are defined as patients who present with the following signs:

- Respiratory rate above 30 breaths per minute
- Fever (temperature >39 °C)
- Heart rate below 120 beats per minute
- Inability to walk unaided and having a low BMI (<18.5) and/or being severely underweight or wasted.

THE LF-LAM TEST SHOULD BE PERFORMED ON SERIOUSLY ILL PATIENTS WITH SUSPECTED TB.

5.4 OUTPATIENTS

By contrast, outpatients (ambulatory patients seen in community health care centres, clinics, hospital outpatient departments and ART initiation clinics) should only be tested with LF-LAM when:

- TB is suspected based on symptoms and/or signs AND
- CD4 counts are ≤200 cells/μL

Xpert should be performed concurrently with the LF-LAM test for all presumptive TB cases who are HIV-positive. However, it should be noted that the Xpert in these patients serves as an additional test to confirm susceptibility or resistance to Rifampicin. In cases where patients can produce a sputum sample, but it is found to be Xpert negative, a culture, drug susceptibility test and LF-LAM test should also be performed.

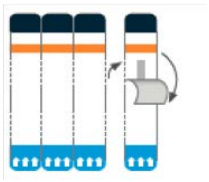
6. Roles and Responsibilities

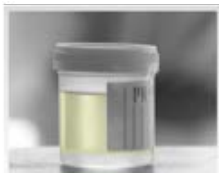
PROFESSIONAL HEALTH CARE PROVIDERS	DUTIES AND RESPONSIBILITIES
• REGISTRARS • SPECIALIST DOCTORS • MEDICAL OFFICERS • COMMUNITY SERVICES DOCTORS • MEDICAL INTERNSHIP DOCTORS • CLINICAL ASSOCIATES	• To ensure all presumptive TB clients who are HIV-positive and/or are seriously ill, HIV-positive patients with a CD4 count of ≤200 cells/μL, and who are unable to produce sputum are screened for TB using LF-LAM. • In cases where HIV patients can produce a sputum sample, but it is Xpert negative, a LAM test should also be done. • Professional health care providers are charged with administering, interpreting and providing appropriate clinical management. • Perform quality assurance measures to ensure accurate application, interpretation and recording of results.
• PROFESSIONAL NURSES	Operational/Unit Managers are responsible for ordering the test kit and are responsible for administering, interpreting and providing appropriate clinical management. The nursing category further includes all categories of nursing staff irrespective of rank who are trained to perform a urine test in any clinical setting. This also includes, but is not limited to enrolled nurses, NIMART and NIMDR-TB nurses.
• PHARMACISTS/PHARMACY ASSISTANTS	Issue and distribute LF-LAM test kits and ensure appropriate storage of test kits.
• HIV AND TB PROGRAMME MANAGERS	• Forecast the volumes of LF-LAM test kits required for district and subdistrict level and monitor demand and supply on an ongoing basis. • Develop a provincial budget in consultation with districts, subdistricts, and other health programmes to monitor the availability of LF-LAM test kits. • Ensure adherence to clinical governance.
• REGIONAL TRAINING CENTRES	Support the LF-LAM roll-out and training at district and subdistrict level.

The target audience for this guidance includes national and provincial policymakers, clinicians, professional health care providers, HIV and TB district and provincial coordinators, as well as technical and implementing partners supporting the use of TB diagnostics in resource-limited clinical settings. This document provides a description of duties for each professional health care provider in ensuring optimal service delivery.

7. Materials and Equipment Required

- Determine™ TB LAM Ag test kit
- A sterile specimen collection container
- Disposable gloves
- Blotting paper sheet
- Bulb pipette (60 µL) provided with test kit
- Biohazard medical waste bin
- Timer (to monitor the 25 minutes required to generate results)




8. Procedure for Performing the Test

8.1 PRETESTING PHASE

- Prepare testing station by ensuring all materials are neatly set out.
- Check the condition of test kit and strips, record kit lot number, confirm the expiry date and storage conditions of the kit.
- The tests should not be used if the packaging is damaged or tests strips are wet.
- The foil pouch with remaining tests together with the desiccant must be resealed immediately after removal of a test strip, by pressing the seal from end to end to close.
- Ensure adherence to infection prevention and control measures. Collect the urine specimen according to the guidance outlined in this document. Clear instructions should be provided to the patient on the collection of urine.
- It is desirable, though not essential, to collect a first or early morning urine specimen as this increases the yield considerably.

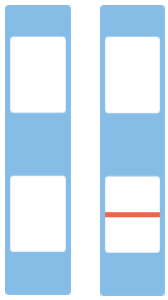
8.2 TESTING PHASE

8.2.1 PERFORMING THE TEST

- The LF-LAM is a strip test which is performed on unprocessed urine. Using pipette provided, add 60 µL (0.06 mL) of urine to the sample pad of the test strip.
- Wait a minimum of 25 minutes and read the result. Visualise the strip under standard indoor lighting conditions or in the shade. Do not visualise the strip under direct sunlight. Results are stable for up to 35 minutes after sample application. Do not read beyond 35 minutes.

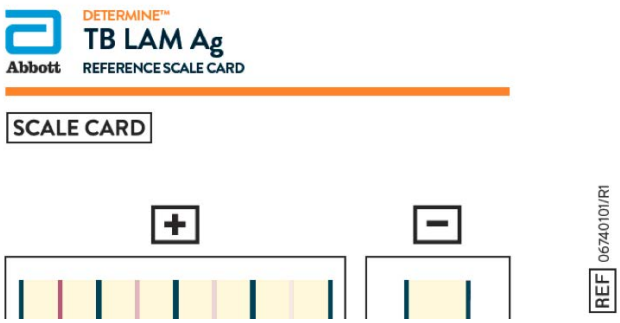
8.2.2 INTERPRETING THE TEST RESULTS

The strip is then inspected with the naked eye in standard indoor lighting. To assist with the reading and interpretation of the results, the reference scale card (provided in the kit) should be used. This is held alongside the window labelled Patient on the test strip, and the intensity of any visible band on the test strip is compared with those on the reference card.

LINE	POSITIVE	NEGATIVE	INVALID
CONTROL			
PATIENT			

8.3 AID FOR RESULT INTERPRETATION

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



8.3.1 TEST RESULTS AND INTERPRETATION: POSITIVE

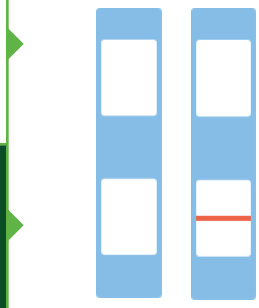
LINE	POSITIVE	LAM ANTIGEN POSITIVE
CONTROL		(Two bars – Control and Patient Bars) Purple/grey bars appear in both the control window (labelled "Control")
		AND
PATIENT		The Patient window (labelled "Patient") of the strip. Colour intensity should be equal to or stronger than any of the coloured bars in the positive range on the reference scale card.

Note: The test result is positive even if the patient bar appears lighter or darker than the control bar. This means that so long as there is a control line present it is a valid test even if the control line is lighter than the patient line. There is no correlation between the control line and patient line.

8.3.2 TEST RESULTS AND INTERPRETATION: NEGATIVE

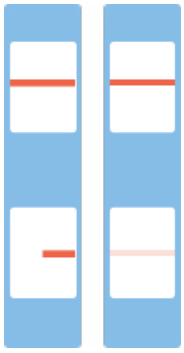
LINE	NEGATIVE	LAM ANTIGEN NEGATIVE
CONTROL		(One bar – Control Bar) One purple/grey bar appears in the control window of the strip (labelled "Control")
PATIENT		No purple/grey bar appears in the Patient window of the strip (labelled "Patient").

8.3.3 TEST RESULTS AND INTERPRETATION: INVALID

LINE	POSITIVE	LAM ANTIGEN INVALID
CONTROL		(No Control Bar) 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.
PATIENT		If the problem persists, contact Technical Support or your local Abbott representative.

Note: The test result is positive even if the patient bar appears lighter or darker than the control bar. This means that so long as there is a control line present, it is a valid test even if control line is lighter than the patient line. There is no correlation between the control line and patient line.

8.3.4 TEST RESULTS AND INTERPRETATION: EQUIVOCAL/INDEFINITE

LINE	NEGATIVE	LAM ANTIGEN EQUIVOCAL/INDEFINITE
CONTROL		(One bar – Control Bar) One purple/grey bar appears in the control window of the strip (labelled “Control”) with unclear or incomplete (broken line or dot) purple/grey bar in the Patient window of the strip (labelled “Patient”).
PATIENT		**OR** The colour intensity of the bar in the Patient window is lower than coloured bars in the Positive range on the reference scale card. 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. First or early morning urine is recommended.

8.4 DISPOSING OF SPECIMENS

- Discard the urine.
- Dispose of the test strip, pipette, and gloves in the biohazard medical waste bin for disposal in accordance with institutional waste disposal guidelines and national or local waste disposal procedures for hazardous waste.

9. Storage Requirements

KIT STORAGE

- The tests are available in packages of 25 test strips and include a reference scale card and a desiccant. The shelf life for the test strips is **17 months from manufacture.** Expiry is indicated on the pouch and each test card. **DO NOT USE BEYOND THE EXPIRY DATE.**
- The storage conditions and room temperature must be checked daily. In hot areas, the test may be stored in the refrigerator – **DO NOT FREEZE.**

SAMPLE STORAGE

- 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.
- Bring all refrigerated urine samples to room temperature one hour before use.
- If testing is delayed for more than 3 days, urine samples should be frozen (–20 °C or colder).
- Refer to instructions for use for further information on frozen samples.

10. Quality Control

10.1 PROCEDURAL QUALITY CONTROL

The test has an internal quality control mechanism which is reflected in the window labelled Control on the test strip. A purple/grey band in this window indicates a valid test. The test result must be read in conjunction with the reference scale card supplied with the kit to minimise false positive and negative results.

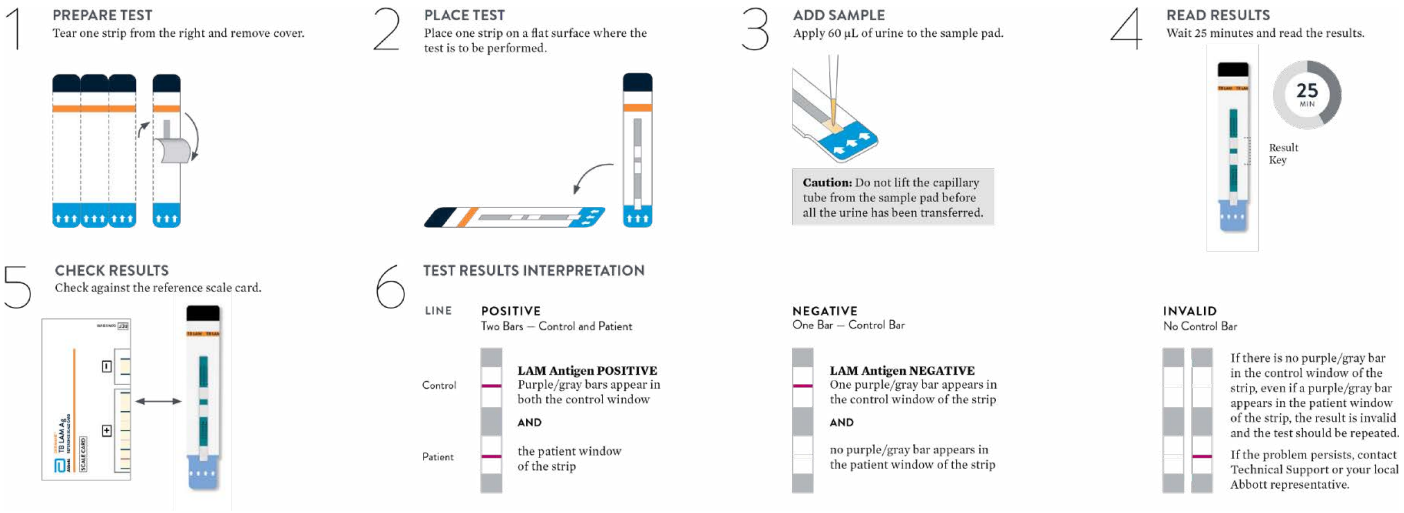
- The tests should not be used if the packaging is damaged or tests strips are wet.
- The foil pouch with remaining tests together with the desiccant must be resealed immediately after removal of a test strip, by pressing seal from end to end to close.

POSSIBLE CAUSE/INTERPRETATION		ACTION
NO BAND IN THE CONTROL WINDOW	• Insufficient waiting period • Insufficient urine specimen • Damaged or faulty test strip • Expired test strip	Repeat the test using another test strip
EXTREMELY FAINT BAND IN THE CONTROL WINDOW	• The control line can vary in intensity. Any visible line validates the results	No action required

- 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.
- If testing multiple samples at a time there is no need to separate the strips; just label them appropriately.
- The assay should be initiated immediately after removing the protective foil cover from each test.

10.2 QUALITY CONTROL REQUIREMENTS

This guidance document recommends periodic assessment of quality control recommendations for health care workers to ensure accurate specimen application and interpretation. Quality control measures can be performed by the attending health care worker and this process should be guided by the recommendations outlined in this document and on the instruction sheet. Follow the steps in the application manual to evaluate the accuracy of the test kit using positive and negative controls.

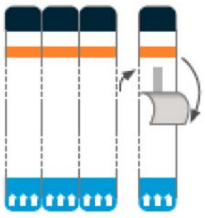


11. Step-by-step Guide on the Use of the Test

1. Advise patient to:

- Clean and wipe dry the urogenital area before collecting urine.
- Allow the first stream of urine to flow and collect midstream urine into the container provided.
- Wipe the external sides of the container and wash hands after collecting urine.

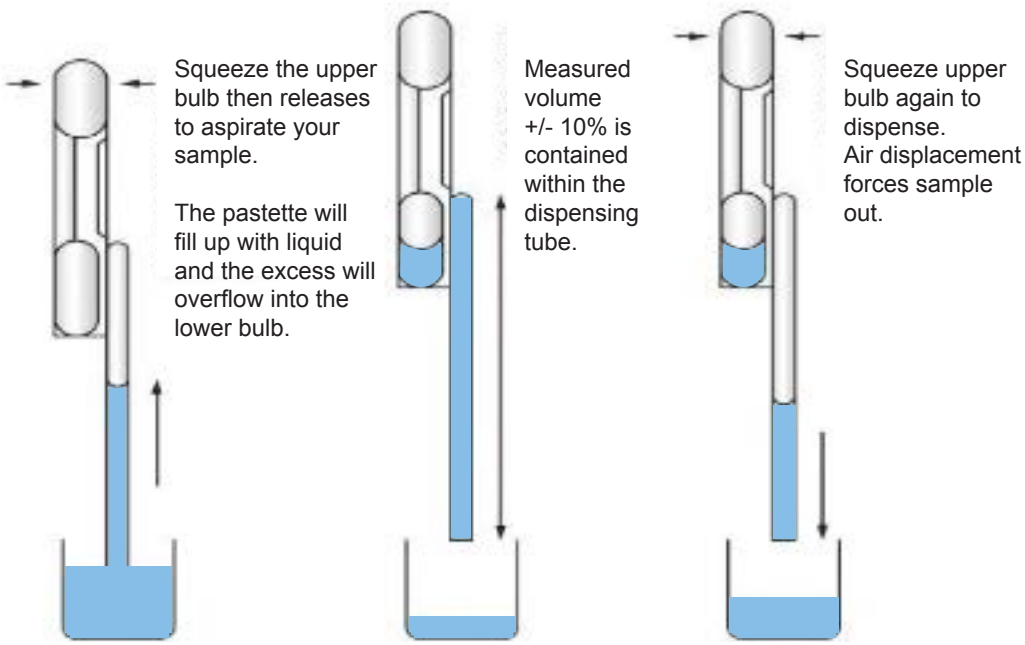
AN EARLY MORNING URINE SPECIMEN IS RECOMMENDED.



2. Tear off one strip at the perforation starting from the right side of the card.
3. Remove the protective foil cover to expose the test strip.
4. Using pipette provided, add 60 µL of urine into the white pad marked by the arrows.

WAIT FOR 25 MINUTES AND THEN READ THE STRIP.
DO NOT READ AFTER 35 MINUTES

HOW TO USE THE PIPETTE SUPPLIED



12. Reading and Interpretation of Test Results

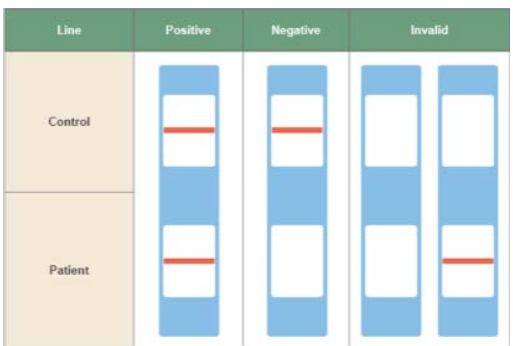
REFER TO SECTION 8 FOR TEST INTERPRETATION

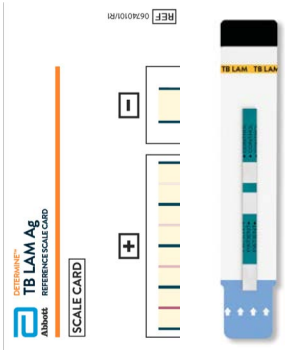
1. Check if there is a band visible in the control window of the test strip.

2. If there is a band visible in the control window, then check the patient window for a visible band
- If a band is visible in this window, the test is positive (provided it meets the criteria in step 3 below).
- If there is no band in this window, the test is negative.
- If the band in this window is incomplete or not clear, the test is indefinite. Repeat the test on another urine specimen, preferably an early morning urine sample.

3. To assist with the reading, match the intensity of the band in the patient window of the test strip against the reference scale card to determine the positivity.
- If the intensity of the band in the patient window is lower than any of the coloured bands in the section marked positive in the reference scale card, the test is indefinite/equivocal and must be repeated on another sample of urine.
- Preferably, an early morning urine sample should be used.

4. A log should be kept of all tests performed for LF-LAM together with the patient details and folder numbers as a record for future reference and in case results are required by another centre or during admission.





THE ALGORITHM APPLIES TO ALL HIV-INFECTED PEOPLE WITH PRESUMPTIVE TB WHO ARE EITHER HOSPITALISED OR ARE AMBULATORY

health
Department:
Health
REPUBLIC OF SOUTH AFRICA

NDP 2030

SETTING	ADMITTED, HOSPITALISED SETTING	CLINIC, OUTPATIENT SETTING, AND PATIENT AMBULATORY AND WITH SIGNS AND SYMPTOMS OF TB
STEP 1 Assess for TB signs and symptoms	All hospitalised patients are considered seriously ill. Proceed with collection of urine for LF-LAM.	**If CD4 count is ≤200 and results are not older than 6 months, perform LF-LAM.** If no CD4 count is available, this should be requested. Patients who have advanced disease and/or are seriously ill should receive LF-LAM. **IF CD4 >200, patient is not eligible for LF-LAM; refer for TPT/ART/Bactrim.**
STEP 2 Perform LF-LAM test. Where possible, collect sputum and perform GeneXpert concurrently.	1. Collect urine for LF-LAM test 2. Collect sputum or any suitable specimen (e.g. CSF, lymph node aspirate, etc.) for testing on GeneXpert Ultra 3. Conduct additional clinical evaluations for TB (initiate treatment with broad-spectrum antibiotics but not fluoroquinolones for bacterial infections, consider treatment for Pneumocystis pneumonia, conduct chest X-ray (CXR) if available)	
STEP 3 Interpret LF-LAM Result	**LF-LAM POSITIVE** **Start patient on TB treatment (first-line treatment). Review GeneXpert Ultra Result**	**LF-LAM NEGATIVE** **Review GeneXpert Ultra result**
STEP 4 Review GeneXpert Result to determine clinical management response	If GeneXpert-positive, review resistance pattern **MODIFY TREATMENT REGIMEN ACCORDING TO GENEXPERT ULTRA RESULTS** If Rifampicin-resistant, follow MDR-TB treatment and clinical guidelines (2019)	**If GeneXpert-negative, consider one or more of the following options:** 1. Send sputum/non-sputum sample for TB culture and DST to rule in DR-TB. Culture and DST should be requested whenever biological samples can be obtained (if not initially available) and appropriate specialist consultation should be sought if there is poor response to TB treatment. 2. Commence antibiotics for bacterial chest infection if appropriate. 3. Perform CXR and, in inpatients, abdominal ultrasound – start empiric TB treatment if these are suggestive of TB. 4. Consider PCP if dyspnoea is a prominent symptom, if respiratory rate >30/min, or if CXR shows an interstitial infiltrate. 5. Defer treatment while awaiting cultures if patient is stable and radiological investigations do not suggest TB. Ensure patient follow up.	If GeneXpert-positive, review resistance pattern **MODIFY TREATMENT REGIMEN ACCORDING TO GENEXPERT ULTRA RESULTS** If Rifampicin-resistant, follow MDR-TB treatment and clinical guidelines (2019)

KEY DEFINITIONS

Advanced HIV disease (AHD) is defined as a CD4 cell count of fewer than 200 cells/μL or a WHO clinical Stage 3 or 4 at presentation for care. All children with HIV who are aged under 5 years should be considered as having AHD at presentation. However, it should be noted that children under 5 years who are stable on ART should not be classified as having AHD.

Seriously ill patients (adult norms) are defined based on four danger signs: respiratory rate of more than 30 breaths per minute, a temperature of more than 39 °C, heart rate of more than 120 beats per minute, inability to walk unaided and a low BMI (<18.5) and/or severely underweight or wasted.

For children, signs of serious illness include lethargy or unconsciousness, convulsions; unable to drink or breastfeed; and repeated vomiting. Other clinical conditions such as body temperature ≥39 °C and age-defined tachycardia and/or tachypnoea can be considered based on clinical judgement (WHO 2017, Guidelines for managing AHD and rapid initiation of ART).

13. Recording of U-LAM Test Data

- Record the date for LF-LAM specimen collection and results (Positive / Negative) in the patient file. This is the responsibility of the nurse who collects the specimen and it must be done daily.
- Record the date for LF-LAM specimen collection and results (Positive / Negative) in the TB Identification register (GW/13 version 2020) under non-bacteriological investigations conducted section. This is the responsibility of the nurse and it must be done daily.
- LF-LAM test data must be captured into the TB Module in Tier.NET (TB Identification module), using the TB Identification register as a source document for capturing. This is the responsibility of the data capturer and it must be done daily.
- At the end of the month, the clinician must collate data for the LF-LAM tests done with their respective results and complete the monthly summary sheet attached at the back of the TB ID register.

Please Note: For more detailed information, refer to the developed TB Monitoring and Evaluation Standard Operating Procedures.

14. Conclusion

This document guides the use of the lateral flow lipoarabinomannan assay (LF-LAM) for the diagnosis and management of active TB in PLHIV. Users should note that the landscape is rapidly changing, and further guidance and updates will follow in due course.

Annexure 1: ICD10 Codes Summary for Extrapulmonary TB

EXTRAPULMONARY TB	
A17.0	TB MENINGITIS
A17.1	MENINGEAL TUBERCULOMA
A18.0	TB OF BONES AND JOINTS
A18.1	TB OF THE GENITOURINARY SYSTEM
A18.2	TUBERCULOUS PERIPHERAL LYMPHADENOPATHY
A18.3	TB OF INTESTINES, PERITONEUM AND MESENTERIC GLANDS
A18.4	TB OF SKIN AND SUBCUTANEOUS TISSUE
A18.8	TB OF OTHER SPECIFIED ORGANS
A19.0	ACUTE MILIARY TB OF A SINGLE SPECIFIED SITE
A19.1	ACUTE MILIARY TB OF MULTIPLE SITES
A19.2	ACUTE MILIARY TB, UNSPECIFIED
B20.0	HUMAN IMMUNODEFICIENCY (HIV) DISEASE RESULTING IN TB
P37.0	CONGENITAL TB
J65	SILICO-TUBERCULOSIS
J65	PNEUMOCONIOSIS ASSOCIATED WITH TB
A16.3	TB OF INTRA-THORACIC LYMPH NODES, WITHOUT MENTION OF BACTERIOLOGICAL OR HISTOLOGICAL CONFIRMATION
A16.4	TUBERCULOSIS OF THE LARYNX, TRACHEA AND BRONCHUS, WITHOUT MENTION OF BACTERIOLOGICAL OR HISTOLOGICAL CONFIRMATION
A16.5	TUBERCULOUS PLEURITIS, WITHOUT MENTION OF BACTERIOLOGICAL OR HISTOLOGICAL CONFIRMATION
A16.7	PRIMARY RESPIRATORY TB WITHOUT MENTION OF BACTERIOLOGICAL OR HISTOLOGICAL CONFIRMATION
A16.8	OTHER RESPIRATORY TUBERCULOSIS, WITHOUT MENTION OF BACTERIOLOGICAL OR HISTOLOGICAL CONFIRMATION
A16.9	RESPIRATORY TUBERCULOSIS UNSPECIFIED, WITHOUT BACTERIOLOGICAL OR HISTOLOGICAL CONFIRMATION

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