

Practical implementation of lateral flow urine lipoarabinomannan assay (LF-LAM) for detection of active tuberculosis in people living with HIV



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FOREWORD

This practical guide aims to help countries implement and incorporate the Alere Determine™ TB LAM Ag (antigen) assay into their routine diagnostics algorithm for tuberculosis (TB). It contains new evidence collected through many studies conducted after the first World Health Organization (WHO) policy on the use of the lateral flow urine lipoarabinomannan assay (LF-LAM) was released in 2015 and then updated in November 2019.

LF-LAM will assist in diagnosing active TB among adults, adolescents and children living with HIV who:

- present to inpatient care facilities with signs and symptoms of TB or with advanced HIV disease or who are seriously ill; or irrespective of signs and symptoms of TB and with a CD4 cell count of less than 200 cells/mm³ in inpatient settings;
- present to outpatient care facilities with signs and symptoms of TB or who are seriously ill; or irrespective of signs and symptoms of TB and with a CD4 cell count of less than 100 cells/mm³ in outpatient settings.

This guide incorporates new recommendations on adding the test to diagnostic algorithms, taking into consideration specific criteria for testing in inpatient and outpatient settings, and it also contains more details on the laboratory process for conducting the assay and on lessons learned, which will help guide countries towards faster and easier implementation of the test.

Despite WHO endorsing the use of the LF-LAM assay and reinforcing its recommendations in 2019, adoption and uptake of the assay has been slow, particularly in settings with high burdens of TB and HIV.

The Global Laboratory Initiative hopes that this practical guide helps implementers understand how the LF-LAM assay works and encourage countries to introduce this point-of-care test into routine care for people living with HIV in order to make the best use of tools to reduce the underdiagnosis of TB in people living with HIV and, thus, reduce overall mortality.

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ABBREVIATIONS

AFB	acid-fast bacilli
Ag	antigen
AHD	advanced HIV disease
AlereLAM	Alere Determine™ TB LAM Ag assay
ART	antiretroviral therapy
CrAg	cryptococcal antigen
CXR	chest X-ray
DR-TB	drug-resistant TB
FIND	Foundation for Innovative New Diagnostics
GDF	Global Drug Facility
GLI	Global Laboratory Initiative
HCW	health care workers
HIV	human immunodeficiency virus
LAM	lipoarabinomannan protein
LAMP	loop-mediated isothermal amplification
LF-LAM	lateral flow lipoarabinomannan assay
mWRD	molecular WHO-recommended rapid diagnostic
MDR-TB	multidrug-resistant tuberculosis
MTB	<i>Mycobacterium tuberculosis</i>
NTP	national tuberculosis programme
PCR	polymerase chain reaction
TB	tuberculosis
WHO	World Health Organization
WRD	WHO-recommended rapid diagnostic

TARGET AUDIENCE

The target audience for this practical guide includes national and subnational policymakers, front-line health workers, managers of HIV and TB programmes, and key TB stakeholders, including implementing partners.

INTRODUCTION

Tuberculosis (TB) is the number one cause of human death worldwide from a single infectious agent, *Mycobacterium tuberculosis* (MTB), ranking above HIV/AIDS. It is estimated that approximately 2 billion people, or 25% of the world's population, are infected with MTB, and 5–10% of infected individuals have a lifetime risk of progressing from TB infection to TB disease (1). The latest global TB report estimated that about 10 million people developed TB disease in 2019. The probability of developing TB disease increases proportionally in the presence of specific risk factors or comorbidities, such as HIV infection. According to the World Health Organization (WHO), in 2017 the risk of developing TB for the 38 million people living with HIV was 18 times higher than the risk for the rest of the global population (1). In 2019, a total of 456 426 cases of TB were notified among HIV-positive people and about 208 000 deaths from TB were reported in the same population. Owing to recent advances in diagnosis and treatment, the past decade has witnessed a global decline in the number of cases of TB and a decline in deaths among individuals coinfecting with TB and HIV. However, there remain serious gaps in and challenges to the early identification of all cases of TB among HIV-positive individuals. In 2015, a systematic literature review of the burden of TB, identified through postmortem studies, showed that TB remains the most important opportunistic infection among this population (2). TB diagnosed at autopsy accounted for almost 40% of HIV-related facility-based deaths in adults (2). This estimate was higher than the WHO–UNAIDS estimate, which was that TB accounted for 25% of the HIV- and AIDS-related deaths worldwide. In 2019, of the 815 000 estimated HIV-associated cases of TB worldwide, only 56% were notified, probably in part due to underdiagnosis and suboptimal access to health care services (1).

Improving access to early and rapid diagnosis of TB is one of the paramount principles in the fight against TB disease and a key pillar of the WHO End TB Strategy (3). Early diagnosis of TB among HIV-positive people is often challenging because the clinical manifestations of TB disease in the presence of HIV infection largely depend on the level of immunosuppression (4).

Conventional sputum smear microscopy tests for TB diagnosis have a reduced sensitivity in HIV-positive individuals, especially if they are seriously ill (5).¹ In HIV-positive people, TB commonly does not produce cavitary lung lesions, and sputum can have a low bacillary concentration, leading to a significant proportion of individuals who are smear-negative or unable to produce sputum. In addition, other challenges make TB diagnosis difficult (6), including the atypical clinical presentation of TB as there is a high prevalence of extrapulmonary and disseminated forms of TB in individuals with advanced immunosuppression.

With the advent of rapid molecular technologies, more accurate TB diagnosis has become more accessible to all patients, including patients with smear-negative and HIV-associated TB (7). A variety of nucleic amplification tests, such as polymerase chain reaction (PCR), real-time PCR and loop-mediated isothermal amplification (LAMP), are available for diagnosing TB (8–12). However, the performance of other WHO-recommended rapid diagnostics, such as TB-LAMP, Truenat™ MTB and Truenat™ MTB Plus (Molbio Diagnostics, Goa, India), have not been fully evaluated for adults and children living with HIV (13). Other technologies such as the Xpert® MTB/RIF assay (Cepheid, Sunnyvale, CA, United States) and, more recently, the Xpert® MTB/RIF Ultra assay are recommended by WHO as the initial TB diagnostic test in adults and children, including those suspected of having HIV-associated TB (11, 12). While the Xpert® MTB/RIF assay achieves a pooled sensitivity of 85% in all adults with pulmonary TB, its sensitivity is decreased to 81% in people living with HIV, and its sensitivity is further reduced to 67% in people with smear-negative culture-positive TB (13); however, the Xpert MTB/RIF Ultra assay has a 10% increase in sensitivity in smear-negative culture-positive patients and a 7% increase among HIV-positive individuals.

Although these molecular assays have helped bridge the TB diagnostic gap by achieving more rapid and sensitive test results for detecting TB among people living with HIV, access to timely diagnostics and effective treatment continue to pose challenges. In the majority of countries where TB is endemic, the placement of GeneXpert devices for these assays has been largely targeted to district-level laboratories mostly due to the infrastructure required and the need for a sustainable budget allocation for the procurement and maintenance of diagnostic tools, among others. Therefore, access to the Xpert® MTB/RIF assay at the point of care is restricted (14) in resource-limited settings that have a high burden of TB. Challenges such as ensuring uninterrupted electricity and an adequate laboratory infrastructure, the

¹ A seriously ill adult or adolescent is defined based on four danger signs: respiratory rate > 30 breaths/minute, temperature > 39 °C, heart rate > 120 beats/minute and being unable to walk unaided. A seriously ill child is defined by having any one of the following danger signs: lethargy or unconsciousness, convulsions, being unable to drink or breastfeed, or by repeated vomiting.

use of restrictive diagnostic algorithms and inefficient or insufficient sample referral mechanisms often limit patients' access to rapid molecular testing (15–18). Furthermore, in HIV-positive individuals, the paucibacillary nature of TB and scarce sputum production make the use of sputum-based molecular testing technologies more arduous.

Lipoarabinomannan (LAM) is an immunogenic virulence factor that is released from metabolically active or those that are being degraded and is specific for mycobacteria species (19). LAM was first characterized in 1980 as a potential marker of active TB (19), and it is the most-studied TB biomarker to date. Factors that make LAM an attractive biomarker for TB include that it is derived from and specific to mycobacteria species; it is abundant in the cell wall of MTB; it is heat and protease stable; and it has structural epitopes that are unique to MTB. Additionally, several studies have shown that in TB patients, LAM is found in blood and sputum (20–23) as well as in urine (24, 25). LAM levels in urine are known to be elevated in people with HIV–TB coinfection, and they increase as CD4 counts decrease (26). Mycobacterial antigens in the serum or urine have attracted researchers' interest because this type of TB biomarker does not require collection of a sputum sample and it can be easily measured in low-cost immunoassay-based rapid-test formats. These characteristics led to the development of a lateral-flow LAM assay (LF-LAM), which became commercially available as a rapid test to be used at the point of care, thus allowing mycobacterial LAM to be detected in urine samples.

In 2015, the WHO Global TB Programme convened a guideline development group to review evidence for the use of LF-LAM. The Guideline Development Group recommended using this assay as a simple point-of-care test to assist in diagnosing TB in HIV-positive adult inpatients with signs and symptoms of TB and with CD4 cell counts ≤ 100 cells/mm³ or in HIV-positive people who are seriously ill regardless of their CD4 count or who have an unknown CD4 count (27). This recommendation also applied to HIV-positive adults who are outpatients and have signs and symptoms of TB (pulmonary or extrapulmonary or both) and who have a CD4 cell count ≤ 100 cells/mm³ or who are seriously ill, regardless of their CD4 count, based on the generalization of data from inpatients. This recommendation also applied to children living with HIV who have signs and symptoms of TB (pulmonary or extrapulmonary or both) based on the generalization of data from adults, while acknowledging that data are limited and there are concerns regarding the low specificity of LF-LAM in children.

The evidence and recommendations applied only to the use of the Alere Determine™ TB LAM Ag (antigen) assay (AlereLAM; Abbott Laboratories, Lake Bluff, IL, USA). Additionally, in 2016, WHO also released *Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection*, which included the recommendation to use LF-LAM to diagnose TB in HIV-positive people who have low CD4 counts or who are seriously ill (28).

While the evidence evaluated by the Guideline Development Group supported the use of LF-LAM as a diagnostic test for TB in HIV-positive adults who meet the criteria described above, new evidence led WHO to revise and expand the recommendations on the use of LF-LAM. Therefore, in November 2019, an updated policy was released by WHO (29).

The updated policy (29) for diagnosing active TB in HIV-positive people contains several changes that increase the strength and scope of the previous recommendations, expand the testing criteria and improve the quality of evidence (Box 1). For a summary of the changes made to the 2015 policy in the updated 2019 policy, see Annex 1.

The new systematic review conducted for the updated LF-LAM policy evaluated the accuracy of AlereLAM as a diagnostic test and its impact on patient-important outcomes, such as TB-related mortality (29). The pooled risk ratio for mortality was 85% for patients with advanced HIV disease (AHD). The accuracy was assessed in different groups including people with or without symptoms, based on their CD4 count and on the CD4 count plus the setting for testing (i.e. an inpatient hospital lab or outpatient setting with clinical testing by trained health care workers). Results from this systematic review showed a slight and insignificant difference around the accuracy of the test when compared with the 2015 policy. The overall pooled sensitivity for AlereLAM for HIV-positive adults with signs and symptoms of TB was 42% and the pooled specificity was 91%. However, the assessed evidence showed the significance of the test for a particular group: the inpatient with or without signs and symptoms of TB and a CD4 cell count < 200 cells/mm³. In the 2015 policy, the recommendation was that AlereLAM should be used as a diagnostic aid for people with CD4 counts < 100 cells/mm³ or who were seriously ill; however, the 2019 policy recommends that all HIV-positive inpatients with advanced disease (CD4 counts < 200 cells/mm³) and who are unable to produce sputum can be tested with AlereLAM.

Currently, AlereLAM is the only commercially available and WHO-approved point-of-care rapid urine-based LF-LAM, although other assays are under development. As the assay has the merit of being a simple, rapid and low-cost test that requires no additional instruments or infrastructure, increasing its uptake could positively improve the impact and utility that the test offers for resource-constrained areas where HIV or TB is endemic.

Box 1. Updated WHO policy (2019) on the use of the Alere Determine™ TB LAM Ag (antigen) assay (AlereLAM)

In inpatient settings

WHO strongly recommends the use of AlereLAM (lipoarabinomannan) to assist in the diagnosis of active TB in HIV-positive adults, adolescents and children:

- with signs and symptoms of TB (pulmonary or extrapulmonary) or
- with advanced HIV disease^a or
- who are seriously ill or
- irrespective of signs and symptoms of TB who have a CD4 cell count of less than 200 cells/mm³.

In outpatient settings

WHO suggests using AlereLAM to assist in the diagnosis of active TB in HIV-positive adults, adolescents and children:

- with signs and symptoms of TB (pulmonary and/or extrapulmonary) or
- or seriously ill or
- irrespective of signs and symptoms of TB who have a CD4 cell count of less than 100 cells/mm³.

In outpatient settings

WHO recommends **against** using AlereLAM to assist in the diagnosis of active TB in HIV-positive adults, adolescents and children:

- without assessing TB symptoms
- without TB symptoms and who have an unknown CD4 cell count or
- without TB symptoms and who have a CD4 cell count ≥ 200 cells/mm³ or
- without TB symptoms and have a CD4 cell count of 100–200 cells/mm³.

Remarks

- The reviewed evidence and recommendations apply only to the use of AlereLAM because other in-house LAM-based assays have not been adequately validated or used outside limited research settings. Any new or generic LAM-based assay should be subject to adequate validation in the settings of intended use.
- All patients with signs and symptoms of pulmonary TB who are capable of producing sputum should get a rapid molecular test (i.e. Xpert®, Truenat™ or TB-LAMP [loop-mediated isothermal amplification])^b as their initial diagnostic test, and at least one sputum specimen should be submitted for rapid molecular testing. This advice also includes HIV-positive children and adolescents who are able to provide a sputum sample.
- These recommendations also apply to HIV-positive adolescents and children, based on the generalization of data from adults, while acknowledging the limited data for these population groups.
- AlereLAM should be used as an add-on to clinical judgement in combination with other tests. It should not be used as a replacement or triage test.

^a For HIV-positive adults, adolescents and children aged ≥ 5 years, advanced HIV disease is defined as a CD4 cell count of < 200 cells/mm³ or a WHO clinical stage 3 or 4 event at presentation for care. All children with HIV who are younger than 5 years should be considered as having advanced disease at presentation.

^b There is uncertainty about the use of the Truenat test in HIV-positive people. In smear-negative patients, the sensitivity is lower than it is in all patients. The indirect data on test accuracy in smear-negative patients allowed this recommendation to be extrapolated to HIV-positive patients because there are no data for HIV-positive patients. However, the certainty of evidence for test accuracy would be lowered for additional indirectness. TB-LAMP has not been fully evaluated for use in HIV-positive people.

BASICS OF THE ALERELAM LATERAL FLOW ASSAY

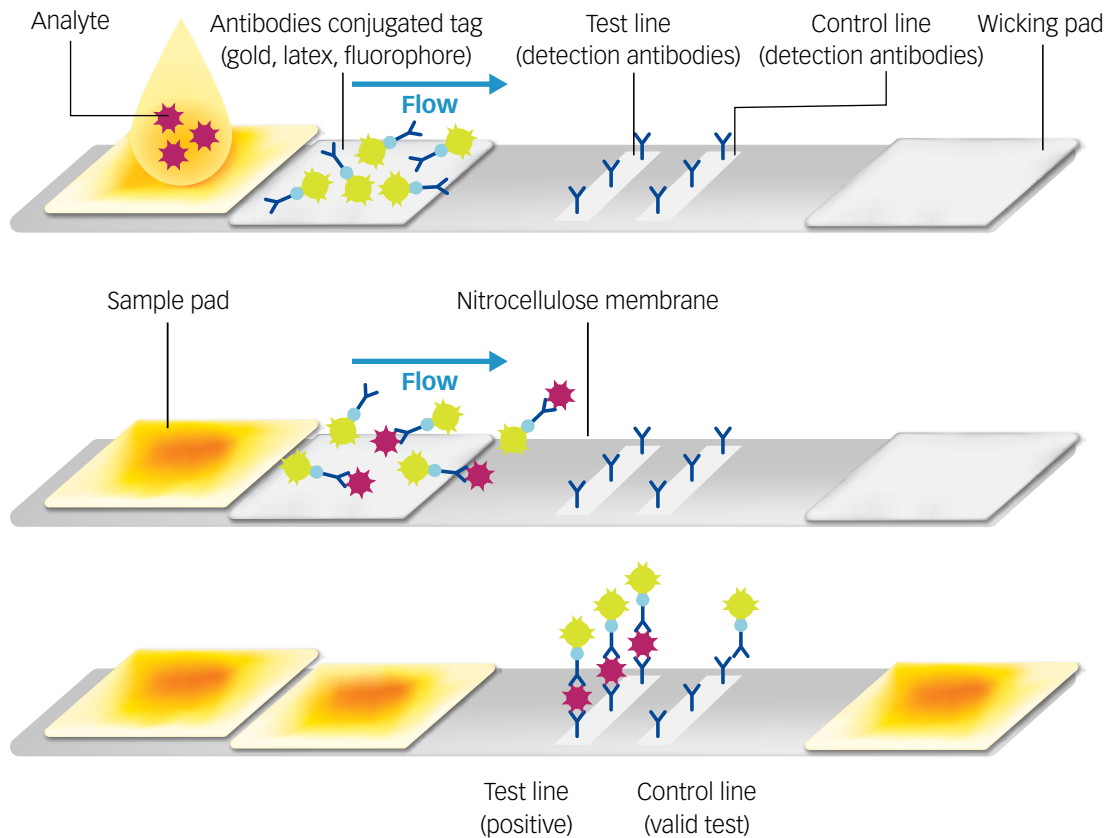
Assay principles

AlerelAM consists of an immunochromatographic assay for the qualitative detection of LAM antigen from mycobacteria in human urine specimens. LAM is a major lipopolysaccharide component of the outer cell wall of mycobacteria. It is a 17.5 kDa glycoprotein found on the surface of the cell. In people living with HIV who are seriously ill, TB can disseminate into various organs. Since LAM is filtered by the kidneys, it is detectable in urine, particularly in patients with AHD and disseminated TB. Finding LAM in urine typically indicates severe disease that requires immediate treatment. In addition, in patients with TB who are immunocompromised, the bacilli are not contained by typical immune responses due to the patient's low CD4 cell level and impaired response; thus, TB bacilli can be degraded and excreted by normal body processes. In both scenarios, the MTB LAM antigen can be present in urine, making detection viable for diagnosis (24, 30).

The test uses highly purified polyclonal antibodies to capture LAM molecules (the target antigen) with a lateral flow, sandwich-based enzyme-linked immunosorbent assay (Fig. 1). The specimen is added to the test strip and capillary flow moves the LAM antigen across the strip so that (a) it binds to a colloidal gold conjugate antibody to form an immunocomplex; (b) capillary flow moves the immunocomplex past the control and patient windows where it is captured by an anti-LAM antibody fixed to the nitrocellulose membrane; and (c) the presence of LAM is confirmed by the colloidal gold label. A purple–grey band in the patient window indicates a positive result, showing that LAM antigen from mycobacteria is present in the sample at or above the detection limit of the test (Fig. 2). If no band is visible, then LAM either is not present or possibly present below the detection limit, and thus the result is presumed to be negative. A control window has been added to ensure the validity of the test; a line should be visible in the control window for every test. The control band uses an antibody with specificity to the colloidal gold (31).

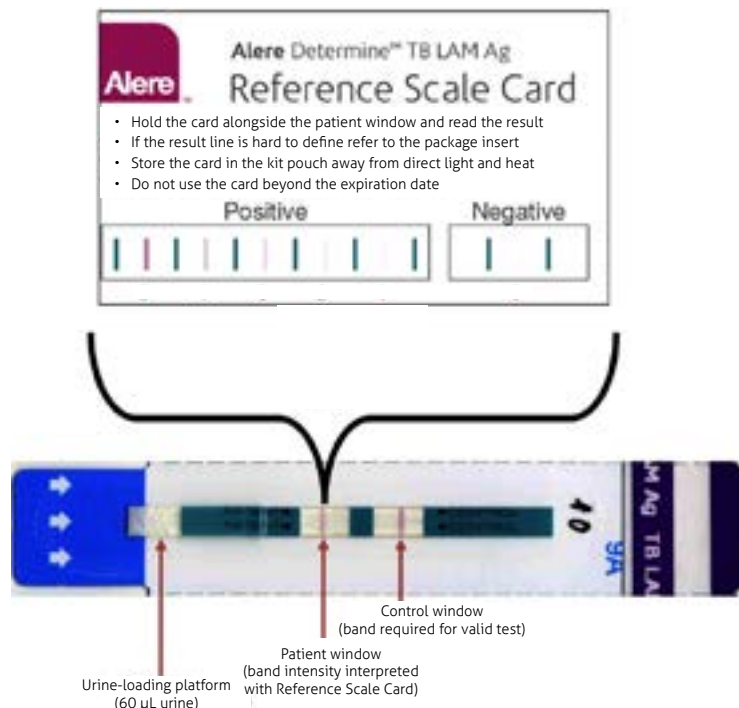
It is important to note that the LAM molecule can be found in all mycobacteria, and thus the test cannot discriminate, for example, between MTB and other species of mycobacteria, including *M. leprae* and *M. avium* (31). However, in areas endemic for TB and in the target population for which WHO has recommended using LF-LAM, a positive result is likely to reflect infection with MTB. Studies have shown that the specificity of AlerelAM varies depending on which test is selected as the reference standard (32). The commonly used reference standard (i.e. culture) has limited performance in the population targeted for the use of this test, due to the population's impaired capacity to produce good-quality sputum samples and the higher proportion of cases with disseminated TB. Thus, the specificity data for AlerelAM need to be interpreted with caution, considering that the test may be able to detect MTB in cases that are missed by the reference standard. Although performing confirmatory TB tests (e.g. a rapid molecular test or culture) is routinely recommended (13, 29) when there are discordant results (i.e. AlerelAM positive and rapid molecular test negative or culture negative), the interpretation of such tests needs to consider the limitations of the Xpert MTB/RIF assay and culture testing of paucibacillary sputum samples (see [Algorithm considerations](#)).

Fig. 1. General principles* for detecting the lipoarabinomannan (LAM) antigen



* Sample containing the analyte of interest moves by capillary action across an internal membrane when applied to the assay where it will bind first to capture antibodies which have a reporter molecule attached. The analyte-antibody complex then continues to migrate until reaching another set of detection antibodies fixed to the membrane which binds the complexed molecules, concentrating them in one place (test line) for detection. Any remaining unbound capture antibodies continue to migrate and complex to a second set of fixed antibodies at a control line which validates the test.

Fig. 2. Using the Reference Scale Card to determine band intensity and validity of the Alere Determine TB LAM Ag assay



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AlereLAM: product specifications

AlereLAM is a lateral flow, rapid diagnostic test with a time-to-result of 25 minutes. AlereLAM is a simple point-of-care test that requires minimal infrastructure and training. The test can be performed in either a laboratory or a clinic. Accurate sample volumes can be obtained by using appropriate delivery devices (see the next section). This allows for testing in clinic settings where there are trained health care staff, thereby enabling task sharing in conducting the test and decentralization of the test to primary health care settings. The test must be stored at 2–30 °C, and it has a shelf-life of 18 months. Kits are stable until their expiration date if stored properly. The test is not recommended by the manufacturer for use with any sample type other than urine.

Supplies needed for testing

The AlereLAM test kit comes with 10 cards containing 10 tests per card, a Reference Scale Card and a package insert with instructions (Fig. 3).

Other items required for testing but not included in the test kit, are a:

- sterile urine collection cup;
- pipette or other device capable of accurately delivering 60 μ L of urine (this could be a calibrated 100 μ L micropipette with filter tips, a dual-bulb 60 μ L micropipette or a Pastette);
- timer.

Fig. 3. The items required for the Alere Determine TB LAM Ag assay include the test card, the Reference Scale Card, a sterile urine collection cup, a pipette and a timer



10 cards (10 tests/card)



Urine collection cup



100 μ L micropipette with tips

or



Dual-bulb 60 μ L micropipette



Timer

Sample collection and storage

It is highly recommended that prior to collecting the urine sample, the urogenital areas are cleaned with a cleansing wipe. Midstream urine should be collected in a sterile, standard urine specimen cup. Whenever feasible, fresh samples should be tested, ideally immediately after collection. If immediate testing is not possible, urine can be stored at room temperature for a maximum of 8 hours or at 2–8 °C for a maximum of 3 days. If stored at 2–8 °C, samples must be brought to room temperature prior to testing. For research purposes, samples can be frozen at –20 °C. However, freezing can cause the formation of uncharacterized precipitates. Thus, unthawed samples require centrifugation at 10 000 g for 5 minutes at room temperature and then a 60 µL aliquot can be drawn from the clear supernatant for testing. Avoid multiple freeze–thaw cycles (i.e. allow only a maximum of three) as the LAM antigen can deteriorate (31). Note that some studies have indicated that urine LAM reactivity disappears in samples stored for 3 years at –20 °C (33). To date, the stability of frozen urine samples has not been compared with the use of fresh specimens (34) for AlereLAM testing.

The manufacturer recommends using early morning urine to ensure optimal test performance. The optimal time for sample collection was studied by Gina et al. in South Africa (35); the study showed that using early morning urine from hospitalized HIV-positive patients increased test sensitivity by 14% in patients with probable TB and by 27% in patients with confirmed TB. Therefore, the time of urine collection should be considered to ensure a clear test result or when performing a retest if the initial test was inconclusive.

Procedure

The basic procedure is outlined in [Fig. 4](#), and the standard operating procedure is outlined in [Annex 2](#). It is important to use the test strip within 2 hours of removing it from the protective foil cover. If more than one sample will be tested, be sure to properly label each test strip so that it can be linked correctly to each patient's sample. The workbench should be cleared of materials not used for testing and cleaned with disinfectant. To ensure the accuracy of the test, it is important to follow an organized workflow for testing and timing.

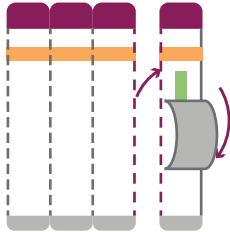
The basic steps are:

- (1) remove the protective foil cover for each test strip needed and ensure they are properly labelled for each patient's sample;
- (2) add 60 µL of urine to the sample pad using a precision pipette or alternative device;
- (3) wait 25 minutes and then read the results. Results are stable for a total of 35 minutes. Do not read after 35 minutes;
- (4) check the results against the Reference Scale card included in the test kit.

Fig. 4. Testing procedure for the Alere Determine TB LAM Ag assay

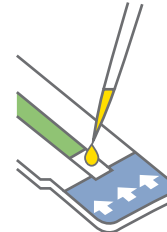
1 Prepare test

Tear one strip from the right side of the test card and remove the foil cover.



2 Add sample

Add 60 µL of urine to the sample pad.



3 Read results

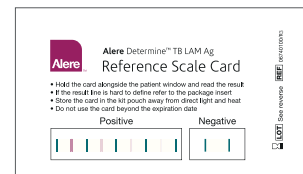
Wait 25 minutes and then read the results.

Line	Reactive	Non-reactive	Invalid
Control			
Patient			



4 Check results

Compare the results with the Reference Scale Card.



Reading and interpretation

A control window acts as an internal quality control measure and is incorporated into the test (Fig. 5). For the test to be valid, a control band must be visible in the control window every time a test is performed. If the control band is not visible, the test is invalid and the sample should be retested. A Reference Scale Card is included in each test kit to assist in interpreting and grading the results (Fig. 2). For a positive test, a purple–grey band should be visible in the control and patient windows. The colour intensity of the patient band should be similar to or darker than any of the positive bands on the Reference Scale Card. The patient and control bands may differ in intensity, but both must be present for a positive test result to be valid. The different colour intensities on the reference card link to a positivity grading, with Grade 1 being the least intense and representing the lowest concentration of LAM. Although this test is not a quantitative test, in general the intensity of the band correlates to the level of LAM in the sample. Low levels of LAM that are near the detection limit can be difficult to interpret.

Importantly, in the current version of the test, a faint band that is lighter than the first positive band on the reference card can appear in the patient window. In this case, the test result should be considered negative or indeterminate. Early implementers of AlereLAM reported that interpreting test results when the band intensity is equal to Grade 1 or lighter than Grade 1 was one of the main operational challenges in using the test. Thus, it is critical to specifically include this topic during training sessions and competency assessments and discuss how to report these types of results (Fig. 6).

Fig. 5. Internal quality control procedure to ensure test validity

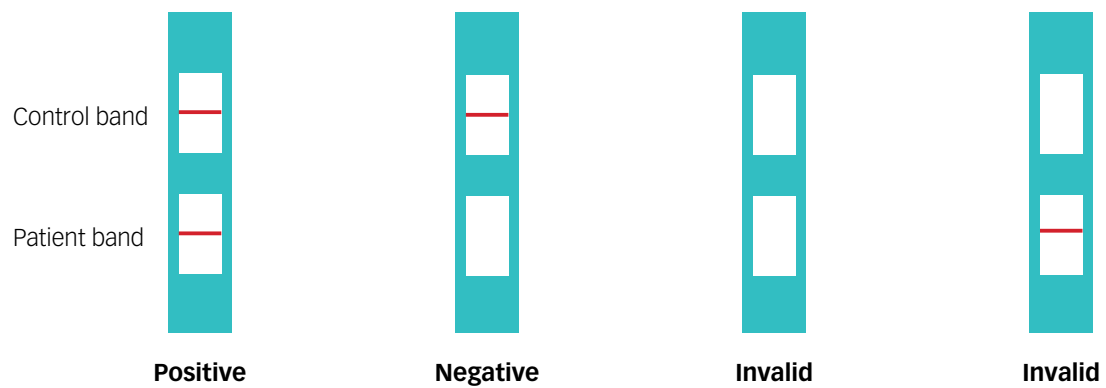
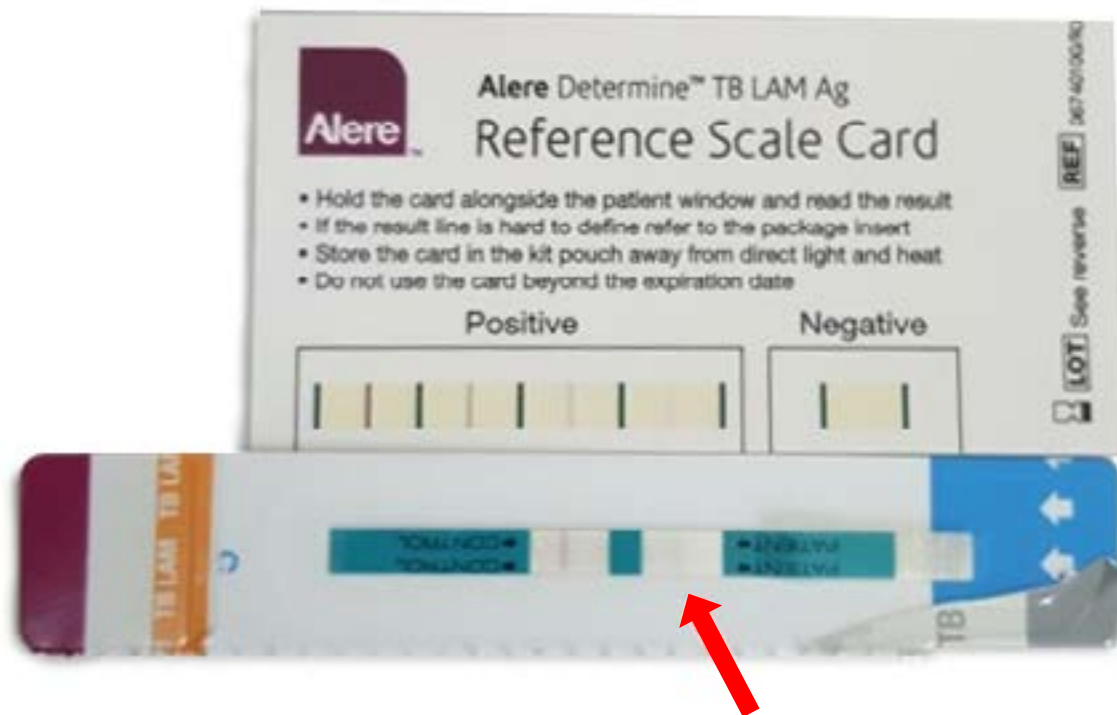


Fig. 6. Example of the unclear or equivocal band intensity sometimes observed with the Alere Determine TB LAM Ag assay that makes it difficult to interpret results



Biosafety best practices and waste disposal

As with all testing of biological specimens, best practices regarding biosafety and waste management should be followed according to each laboratory's biosafety guidelines and standard clinical practice. Personal protective equipment is recommended for handling clinical specimens (e.g. lab coat, gloves). Workspaces for testing should be clean and well organized.

ROLE OF ALERELAM IN DIAGNOSTIC ALGORITHMS FOR TB

In line with the WHO guidelines for managing AHD and for rapidly initiating antiretroviral therapy, LF-LAM has important utility within the package of care that should be offered to people with AHD or HIV-positive people who are seriously ill with any CD4 cell count (36). The package of diagnostic testing for people with AHD should be offered at all levels of the health care system and must include, among others, CD4 cell count, serum cryptococcal antigen (CrAg) testing, LF-LAM and WHO rapid molecular diagnostics for TB. Despite policy recommendations, the extent of implementation of the AHD management package, including roll-out of LF-LAM, is progressing slowly (37).

The revised WHO guidelines on using LF-LAM also include evidence about additional considerations when implementing LF-LAM to diagnose TB among HIV-positive populations, as well as the placement of the test and its value in guiding TB treatment initiation (29). Some of the findings of these studies are summarized below.

- (1) A study conducted in HIV-positive adults in Kenya showed that adding AlereLAM to diagnostic algorithms for managing hospitalized or ambulatory patients who were either seriously ill or had low CD4 counts significantly increased the diagnostic yield, in particular when it was used as an add-on test to algorithms that included only clinical signs and chest X-ray (CXR) (36.6% increase in detection yield) or clinical signs, CXR and smear microscopy (19.9% increase in detection yield) (38). An increase in case detection yield was also observed when AlereLAM was added to a diagnostic algorithm that included the Xpert MTB/RIF assay (13.4% increase in detection yield), showing that using a LAM assay can support TB diagnosis in patients who may be missed by Xpert MTB/RIF testing alone (38).
- (2) A multicountry trial published in 2016 reported that when AlereLAM was added to diagnostic algorithms that included smear microscopy or the Xpert MTB/RIF assay, it played a critical supportive role in shortening the time to diagnosis and time to treatment initiation in HIV-positive patients (39). This is particularly relevant for HIV-positive patients who are at higher risk of death but who are unable to produce sputum for routine diagnostic evaluation (5, 39, 40).
- (3) The same multicountry trial found that AlereLAM contributed to rapid diagnosis and initiation of TB treatment and was associated with a reduction in 8-week mortality in HIV-positive patients with at least one symptom of TB and illness severity necessitating admission to hospital (39).
- (4) Studies, including costing data and cost-effectiveness analyses comparing different diagnostic strategies for TB detection in people living with HIV have indicated that adding a LAM assay to a TB diagnostic algorithm could reduce costs associated with TB diagnosis. The test is highly cost-effective compared with diagnostic algorithms that include either smear microscopy or the Xpert MTB/RIF assay alone in settings with a high burden of TB and HIV, such as Mozambique and Uganda (41, 42).

Algorithm considerations

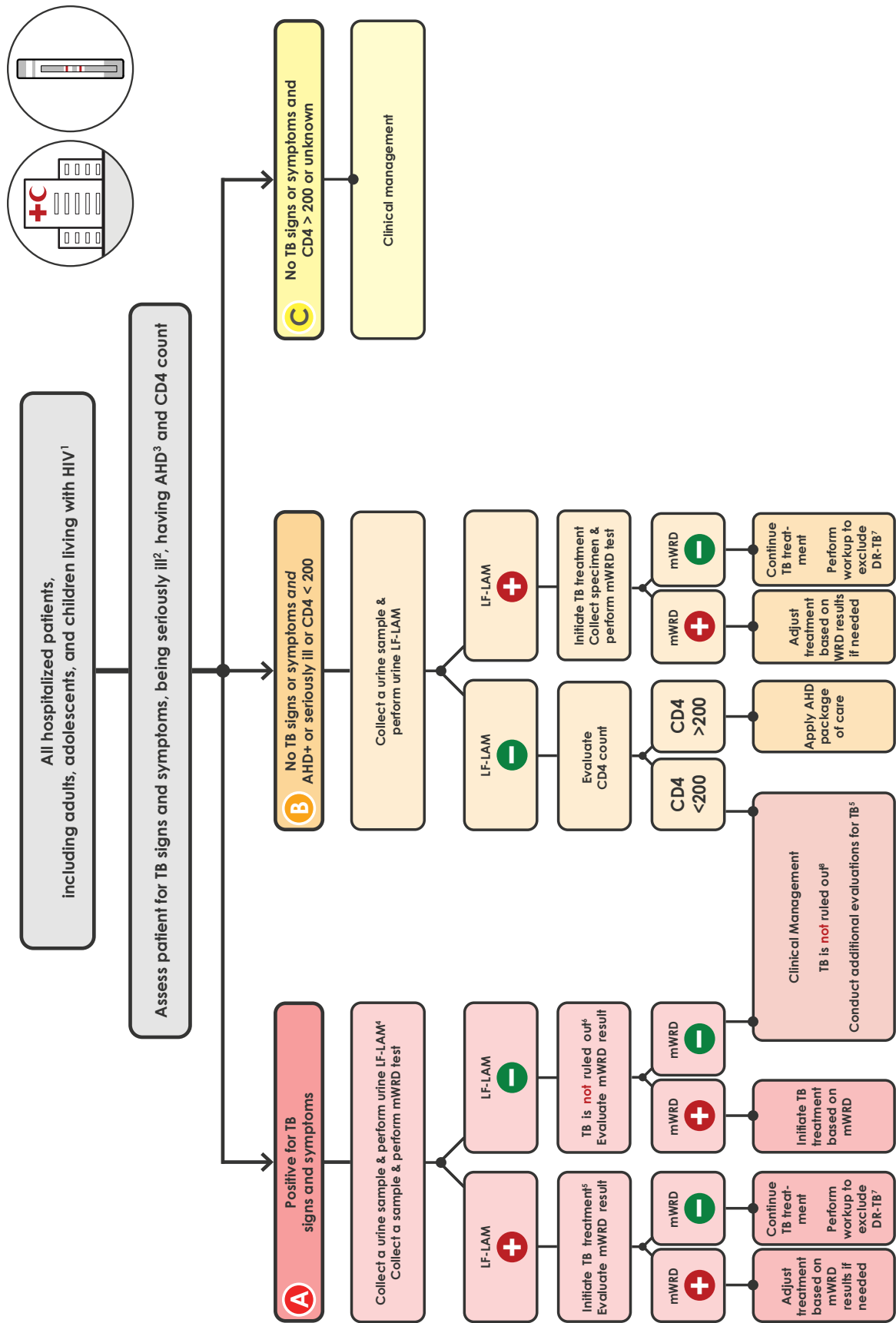
Based on the evidence in the studies mentioned above and on the WHO 2019 recommendations for the use of LF-LAM (29, 43) and on managing care for people with AHD (36), the following practical considerations should inform national algorithms and strategies for rolling out AlereLAM.

- Target populations that should be tested with AlereLAM are:
 - inpatients {
 - HIV-positive patients –
 - with signs and symptoms of TB OR
 - with AHD OR
 - who are seriously ill
 - irrespective of whether there are TB symptoms if they have CD4 counts < 200 cells/mm³;
 - outpatients {
 - HIV-positive patients –
 - with signs and symptoms of TB OR
 - who are seriously ill
 - irrespective of whether there are signs and symptoms of TB if they have a CD4 count < 100 cells/mm³.

- All HIV-positive children aged ≤ 5 years should be considered to have AHD at presentation to the clinic. Therefore, they are eligible for LF-LAM in any setting (inpatient or outpatient), irrespective of whether they have signs and symptoms of TB.
- Molecular WHO-recommended rapid diagnostics should be performed in parallel to LF-LAM in the target populations described above. However, to avoid delays, decisions about immediate treatment should be based on LF-LAM results while awaiting the results of other rapid molecular tests.
- If inpatients are seriously ill or bedridden and cannot produce sputum and are in one of the target populations described above, then AlereLAM can be done at the bedside in order to support the rapid diagnosis and clinical management of patients at risk of TB-related rapid deterioration and death. Clinicians should initiate TB treatment immediately based on a positive AlereLAM result and their clinical judgment while awaiting results of a rapid molecular test or culture-based confirmation, if available.
- Patients with a positive AlereLAM result should immediately be initiated on TB treatment. For a detailed description of considerations for diagnostic follow up, see [Fig. 7](#).

A negative LF-LAM result does not rule-out TB. In the presence of clinical signs and symptoms suggestive of TB, further diagnostic evaluations should be undertaken.

Fig. 7a. Lateral flow lipoarabinomannan assay (LF-LAM) testing to aid in diagnosing TB among HIV-positive people in inpatient settings



Source: WHO operational handbook on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection (43)

AHD: advanced HIV disease; DR-TB: drug-resistant tuberculosis; LF-LAM: lateral flow lipoarabinomannan assay; MDR-TB: multidrug-resistant tuberculosis; mWRD: molecular WHO-recommended rapid diagnostic; TB: tuberculosis; WHO: World Health Organization; WRD: WHO-recommended rapid diagnostic.

1. Persons living with HIV include those who are HIV-positive or whose HIV status is unknown but who present with strong clinical evidence of HIV infection in settings where there is a high prevalence of HIV and individuals at greater risk of becoming infected with HIV. For all people with unknown HIV status, HIV testing should be performed in accordance with national guidelines. HIV-positive people with TB may also present with signs and symptoms of extrapulmonary TB, including lymphadenopathy, meningitis or other atypical presentations that warrant evaluation.

2. Seriously ill is defined based on four danger signs: respiratory rate > 30 breaths/minute, temperature > 39 °C, heart rate > 120 beats/minute and being unable to walk unaided.

3. For adults, adolescents and children aged > 5 years, AHD is defined as a CD4 cell count < 200 cells/mm³ or a WHO stage 3 or 4 disease at presentation for care. All children aged < 5 years are considered as having AHD.

4. The LF-LAM test and mWRD test should be done in parallel. The LF-LAM results (test time < 15 minutes) are likely to be available before the mWRD results; hence, treatment decisions should be based on the LF-LAM result while awaiting the results of other diagnostic tests.

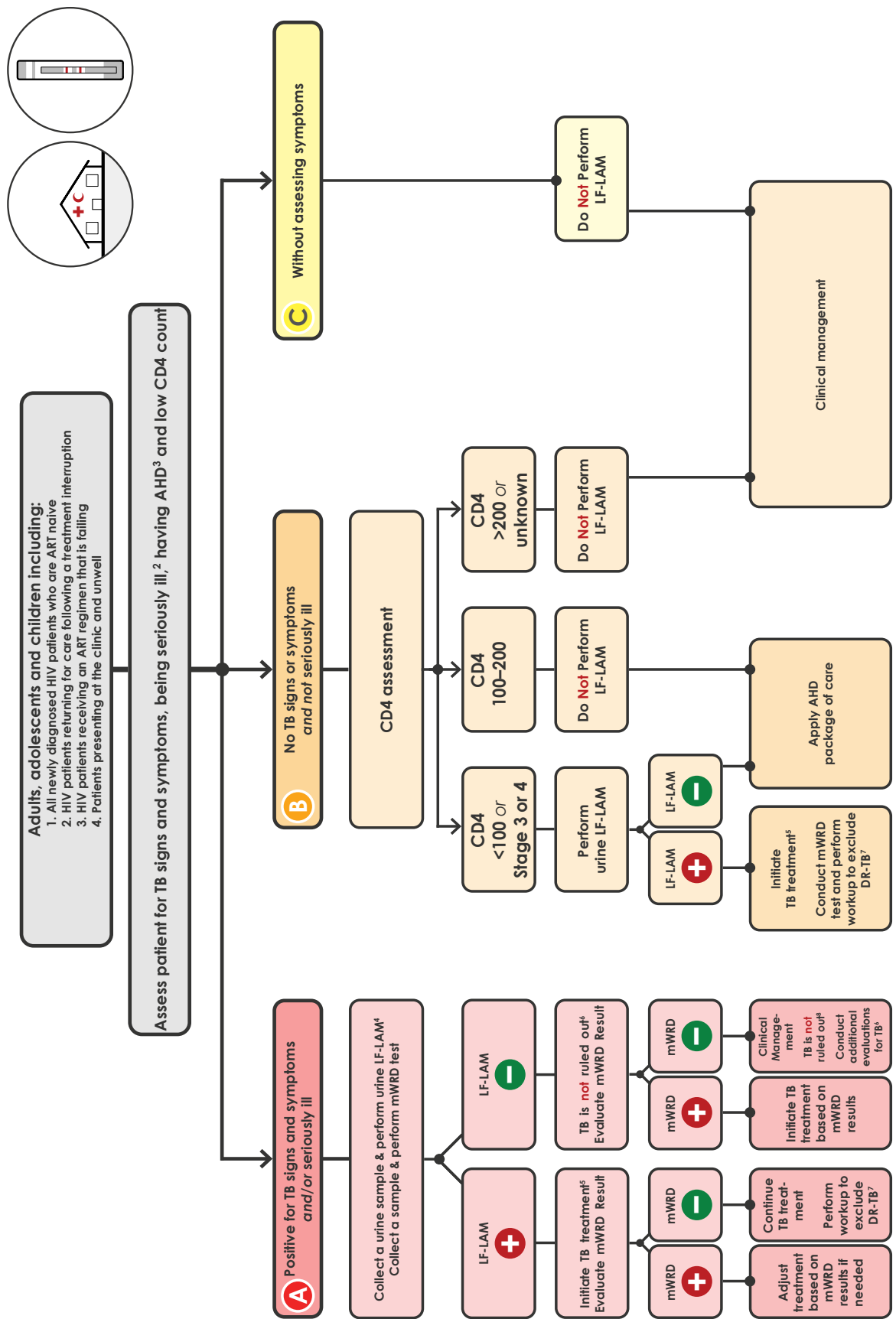
5. Patients should be initiated on a first-line TB treatment regimen according to national guidelines unless they are at very high risk of having MDR-TB. Such patients should be initiated on an MDR-TB regimen.

6. Negative LF-LAM results do not rule out TB in symptomatic persons. The mWRD test result should be evaluated when it becomes available for treatment decisions. See Algorithm 1 in reference 43 for interpretation of molecular WRD results.

7. Phenotypic (culture and drug-susceptibility testing) and molecular (e.g. line probe assays, DNA sequencing and high-throughput assays) methods are available to evaluate drug resistance. Rapid molecular methods (e.g. Xpert® MTB/RIF or Truenat™ MTB diagnostic tests) are preferred.

8. Negative Xpert® MTB/RIF and LF-LAM results do not rule out TB in symptomatic persons. Conduct additional clinical evaluations for TB. Further investigations for TB may include chest X-ray, additional clinical assessments, including clinical response following treatment with broad-spectrum antimicrobial agents, and additional WRD testing or culture. Consider initiating treatment for bacterial infections and for *Pneumocystis pneumonia* using antibiotics with broad-spectrum antibacterial activity (fluoroquinolones should not be used). The clinical response should be evaluated after 3–5 days of treatment.

Fig. 7b. Lateral flow lipoarabinomannan assay (LF-LAM) testing to aid in diagnosing TB among HIV-positive people in clinic and outpatient settings



Source: WHO operational handbook on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection (43)

AHD: advanced HIV disease; ART: antiretroviral therapy; DR-TB: drug-resistant tuberculosis; LF-LAM: lateral flow lipoarabinomannan assay; MDR-TB: multidrug-resistant tuberculosis; mWRD: molecular WHO-recommended rapid diagnostic; RIF: rifampicin TB; tuberculosis; WHO: World Health Organization; WRD: WHO-recommended rapid diagnostic.

1. Persons living with HIV include those who are HIV-positive or whose HIV status is unknown but who present with strong clinical evidence of HIV infection in settings where there is a high prevalence of HIV and individuals at greater risk of becoming infected with HIV. For all people with unknown HIV status, HIV testing should be performed in accordance with national guidelines. HIV-positive people with TB may also present with signs and symptoms of extrapulmonary TB, including lymphadenopathy, meningitis or other atypical presentations that warrant evaluation.
2. Seriously ill is defined based on four danger signs: respiratory rate > 30 breaths/minute, temperature > 39 °C, heart rate > 120 beats/minute and being unable to walk unaided.
3. For adults, adolescents and children aged > 5 years, AHD is defined as a CD4 cell count < 200 cells/mm³ or a WHO stage 3 or 4 disease at presentation for care. All children aged < 5 years are considered as having AHD.
4. The LF-LAM test and mWRD test should be done in parallel. The LF-LAM results (test time < 15 minutes) are likely to be available before mWRD results; hence, treatment decisions should be based on the LF-LAM result while awaiting the results of other diagnostic tests.
5. Patients should be initiated on a first-line TB treatment regimen according to national guidelines, unless they are at very high risk of having MDR-TB. Such patients should be initiated on an MDR-TB regimen. Treatment regimens should be modified as needed based on the results of the mWRD testing.
6. Negative LF-LAM results do not rule out TB in symptomatic persons. The result of the mWRD test should be evaluated when it becomes available (see Algorithm 1 in reference 43 for interpretation of mWRD results).
7. Phenotypic (culture and drug-susceptibility testing) and molecular (e.g. line probe assays, DNA sequencing and high-throughput assays) methods are available to evaluate drug resistance. Rapid molecular methods (e.g. Xpert® MTB/RIF or Truenat™ MTB-RIF diagnostic tests) are preferred. Results of the WRD test should be interpreted as shown in this figure.
8. Negative Xpert® MTB/RIF and LF-LAM results do not rule out TB in symptomatic persons. Conduct additional clinical evaluations for TB. Further investigations for TB may include chest X-ray, additional clinical assessments and additional mWRD testing or culture. Consider initiating treatment for bacterial infections and for *Pneumocystis pneumonia* using antibiotics with broad-spectrum antibacterial activity (not fluoroquinolones). The clinical response should be evaluated after 3–5 days of treatment.

CONSIDERATIONS FOR ROLLING OUT ALERELAM

Country introduction

Prior to implementation and roll-out, countries need to perform a set of specific tasks. First, a formal introduction and overview of the test may be needed for Ministry of Health officials, members of HIV and TB programmes, and civil society. This introduction should outline the test's specifications and limitations, effective placement strategies, test feasibility and utility in specific settings, and the diagnostic impact as reported by other implementers. Depending on their regulatory processes, countries may need to proceed with country registration and validation protocols prior to implementing the test. Forecasting for the number of tests to be procured should be based on annual data about the rates of testing for TB among HIV-positive people, the number of HIV-positive people who are diagnosed with TB and the annual number of cases with AHD (considered to be those with CD4 counts < 200 cells/mm³). Countries should leverage an existing working group or establish a new one to coordinate policies for TB and HIV and define national guidelines for the use and implementation of LF-LAM. Training platforms for clinicians and laboratories will need to be customized to provide information about how to use the test according to algorithms established by both TB and HIV programmes, establish the best practices for urine collection, finalize the protocols for laboratories and health care clinics, guide interpretation of the test and consideration of the limitations of the test, ensure adequate follow-on confirmatory testing, provide information about recording and reporting practices, and support immediate linkage to TB treatment for patients, particularly for those with severe illness. The test will need to be added to request forms as well as to recording and reporting templates to ensure its use for rapid diagnosis. Quality assurance and waste disposal practices will also need to be determined. Efficient and effective implementation will require strong collaboration not only between TB and HIV programmes but also between them and civil society to guarantee a benefit from and have the expected impact on the diagnosis of TB in HIV-positive people and patients who are seriously ill or have AHD.

Based on WHO's 2016 consolidated guidelines on the use of antiretroviral therapy for treating and preventing HIV infection (28) and recommendations on the management of AHD (36), country programmes should also consider that:

Everyone presenting with AHD should be offered a package of care that includes key diagnostic, screening, prophylaxis and adherence interventions.

Implementation considerations

Product registration

The registration process should be initiated early so as not to hinder deployment and use of WHO-endorsed tools. The in-country registration of WHO-endorsed tools should not act as a barrier to introducing LF-LAM, thereby preventing TB diagnosis. If the test has not been officially registered, the Ministry of Health will need to contact the manufacturer to assist with the process. Proper steps to ensure a smooth and efficient process will be essential to rapid implementation of LF-LAM.

In-country validation

In some countries, new products need to undergo country-specific validation studies. Although these are not necessary for WHO-endorsed products that have gone through significant evaluation and expert review, national policies may require them to be completed to assess the performance and feasibility of a new diagnostic test or technology in its specific setting or for a specific population. Countries will need to plan ahead to expedite this activity to ensure an efficient roll-out.

Forecasting and procurement

In order to assess annual procurement needs, both HIV and TB programmes will need to review data on rates of HIV and TB and AHD and stratify patients via CD4 counts to assess the annual percentage of HIV-positive people with CD4 counts < 100 cells/mm³ in outpatient settings and CD4 counts < 200 cells/mm³ in inpatient settings to provide an evidence-based estimate for procurement. These data may be difficult to acquire, but developing a plan to begin annual assessments will allow for more accurate procurement projections that can inform budgeting and planning (see [Procurement information](#)).

Placement of the test

Service delivery points where placement of LF-LAM for TB diagnosis should be considered are:

- for inpatient settings – the test can be placed at level 2 or 3 health care sites or at public or private hospital inpatient wards that have access to laboratory testing performed by workers trained in TB LF-LAM;
- for outpatient settings – the test can be placed at level 1 primary care centres, urgent care clinics and HIV care centres or clinics. Specimen collection and TB LF-LAM would be administered by a trained health care worker.

Document development and revision

Various documents will need to be written or revised to support testing; these documents include:

- national guidelines and algorithms
- strategic plans for implementation
- urine collection protocols
- standard operating procedures and quick guides
- training and competency materials
- request forms, recording templates and reporting forms
- those addressing quality assurance and waste disposal practices.

Recording and reporting

Facilities and national tuberculosis programmes (NTPs) should record and report a positive LF-LAM result as a bacteriologically confirmed TB case. WHO's definitions and reporting framework for tuberculosis, updated in 2020 (44), includes biomarker-based techniques, such as LF-LAM, as approved rapid diagnostic tests able to define a TB case under the conditions established by WHO guidelines (13, 29).

Training staff

Training programmes should include TB and HIV clinical and laboratory staff. As with any new technology being implemented, clinicians need to understand the overall accuracy and limitations of the test, its placement in the diagnostic algorithm, what the results mean and which follow-on tests are needed to ensure quality patient diagnosis and care. Laboratory and clinical staff assigned to perform the test need to fully comprehend the steps and processes associated with sample collection, test application and the visual readout. [Annex 2](#) provides a standard protocol that includes details on how to ensure accurate sample readouts and reliable results. These protocols can be used to develop training materials. On-site training is recommended so that the specifics of each setting can be understood and to ensure user capability and understanding. Half-day training is recommended, and it should be followed by observations of testing the next day. Training components and preparations should include the following:

- a presentation to clinicians and lab staff who will be performing and using the test (e.g. with a slide deck or other training material). The presentation should provide an overview of how the test is used for diagnosing TB among HIV-positive people and include relevant background data demonstrating reduced mortality for the target population, as well as the sensitivity and specificity in the target population. It should highlight studies from other countries. Finally, it should provide information regarding the basics of the test itself, the steps involved in proper specimen collection and the interpretation of test results;

- development and review of bench aids indicating the steps necessary to perform the test and interpret the results;
- hands-on practical testing of known positive and negative samples. If micropipettes are to be used in a clinical setting, then a session on developing pipetting skills is recommended. It is essential to ensure that staff understand the importance of precisely adding the sample to the test strip. It is also essential to ensure that staff understand the correct use of the Reference Scale Card and how to read and interpret the test appropriately, particularly in instances in which the result may be unclear;
- discussion of the test's limitations and best practices for performing the test. This should include information about why the test cannot be used for routine screening of all HIV-positive people;
- information about proper storage and disposal of the test, in accordance with country guidelines and the manufacturer's recommendations;
- definition of the requirements for lot quality assessments for each new shipment of tests as a first step to ensuring quality control;
- inventory management practices that can be used to establish inventory monitoring to define annual consumption rates and monitor expiry dates at each testing site. Further, methods for ensuring proper forecasting and the quantification and communication of needs should be addressed;
- a question and answer session should be offered to address any queries and so that suggestions for improving the training can be made.

Competency assessments to evaluate knowledge and skills should be performed at regular intervals (i.e. at 6 months after initial training and annually thereafter).

Testing in outpatient settings

Test acceptability and demand at the community level are critical to the roll-out of diagnostic tests at primary health care settings. Civil society groups can assist in creating awareness of the test in the community.

Phased introduction

Using a phased approach is the best strategy for implementing any new diagnostic tool. It is recommended that a phased approach begins by placing the test in inpatient settings, where the diagnostic yield will be notable and will significantly reduce mortality. After 6 months, or later, testing can be deployed at lower-level outpatient settings and clinics, particularly at HIV care centres and clinics. In-country data from early inpatient roll-out should be shared with outpatient clinicians to highlight the acceptability of the test and the use of recording and reporting tools.

Lessons learned

Although the majority of the studies conducted with AlereLAM assessed the accuracy of the test, few studies have also quantified the challenges and benefits or successes of implementing AlereLAM through countries' NTP and HIV/AIDS programmes or directly through institutions and facilities in which diagnostic resources are scarce or patients are unable to produce sputum. A survey conducted in countries with high burdens of TB and HIV assessed the uptake of AlereLAM (45). Additionally, the feasibility of routine implementation of AlereLAM has been systematically investigated in operational research studies conducted in different countries (46; Mathabire Rücker SC, Huerga H, Epicentre/Médecins Sans Frontières, unpublished field reports from Democratic Republic of the Congo, Malawi and Mozambique, 2017).

The challenges, successes and lessons learned from the studies above include the following.

Challenges

- Budget limitations: The lack of funding to support uptake of AlereLAM was mentioned by 10 out of 21 (48%) countries with a high TB burden that reported barriers to implementation (44).
- Pilot studies: Some countries reported that it was necessary to conduct pilot studies and evaluations in their country before implementation. These types of studies can inform decision-makers about the best use and placement of the test in national diagnostic algorithms and can also assess accuracy and operational constraints.

- The test is not perceived as a priority: NTPs and HIV/AIDS programmes do not expect to identify high numbers of seriously ill people living with HIV if they have strong programmes in place, including test and treat strategies² (46) and isoniazid preventive therapy. However, sufficient tests should be available for the number of cases forecast based on WHO's new recommendations (Box 1). The number of people living with HIV who would benefit from LF-LAM should be calculated (see [Forecasting orders for rolling out AlereLAM](#)).
- Delays with regulatory approvals for test introduction: Countries may face coordination and administrative limitations for the approvals required to introduce and roll out new medical devices.
- Low sensitivity: Confidence in AlereLAM is low due to its low sensitivity. However, sensitivity increases under specific conditions, such as when it is used for patients with low CD4 counts (24). Therefore, AlereLAM is able to reduce the time to treatment initiation, thus leading to a reduction in overall mortality (Mathabire Rücker SC, Huerga H, Epicentre/Médecins Sans Frontières, unpublished report, Malawi, 2017).
- Limitations at health facilities: Patients need toilets or other clean and private sanitary facilities at health centres to allow them to provide a good-quality sample without the risk of contamination. Additionally, the absence of toilets may cause negative perceptions of the test. A patient may reject testing due to the lack of privacy for urine collection.
- Disturbance to patient flow: It has been reported that for HIV-positive patients who are not classified as seriously ill based on clinical signs and symptoms, a CD4 test needs to be done if previous results are older than 3 months to determine eligibility for AlereLAM. This may translate into a delay for the patient and might possibly require a follow-up visit if the CD4 count and AlereLAM test cannot be done on the same day.

Benefits and successes

The studies conducted in the Democratic Republic of the Congo, Kenya, Malawi and Mozambique had the following conclusions (38, 46).

- Easy to use: AlereLAM was found to be easy to use, requiring minimal training for health workers.
- Shelf-life: The test could be stored at room temperature and had a shelf-life of 1 year.
- No need for laboratory infrastructure: The test could be conducted in the consultation room without any additional laboratory infrastructure, and results were available on the same day as the clinical consultation, offering the possibility of starting TB treatment immediately.
- More patients able to produce urine than sputum: The studies in Kenya and Malawi highlighted that 99% and 100% of patients, respectively, were able to produce urine for testing. In contrast, only about 75% of patient were able to produce sputum for analysis (38; Mathabire Rücker SC, Huerga H, Epicentre/Médecins Sans Frontières, unpublished report, Malawi, 2017).
- Rapid turnaround time: Turnaround time varied from setting to setting. The study in the Democratic Republic of the Congo reported that the median turnaround time for results from AlereLAM was 75 minutes (interquartile range: 45–188) when performed in the consultation room; although the time was shorter in other settings. In comparison, other TB diagnostic tests, particularly the Xpert[®] MTB/RIF assay and microscopy, had a median turnaround time of about 2 days (Mathabire Rücker SC, Huerga H, Epicentre/Médecins Sans Frontières, unpublished report, Democratic Republic of the Congo, 2017).
- High reader agreement: The studies in Malawi and Mozambique found that interreader agreement using the AlereLAM Reference Scale Card was high at 98.3% and 98.9%, respectively.

Lessons learned

- Training: It is essential that training for health workers focuses on identifying and evaluating patients eligible for LF-LAM testing (i.e. implementation of diagnostic algorithms).
- Appropriate disposal of urine samples: It is important to ensure that there is an appropriate place to safely dispose of urine samples in the facility.

² Test and treat is a strategic intervention in which individuals are tested regularly for HIV and provided immediately with antiretroviral therapy if they are HIV-positive, regardless of their CD4 count.

- Additional staff workload: Although the extra workload caused by implementing AlereLAM is minimal, additional time may be required in inpatient departments if there is a need to insert a urinary catheter for collection when the patient is seriously ill.
- Clear educational messages to patients to avoid noncompliance with other TB diagnostic tests: Due to the ease of providing a urine sample for AlereLAM, some patients may be reluctant to allow collection of different types of samples (such as sputum) to perform other TB tests. This can be a critical problem due to the low sensitivity of AlereLAM. Additionally, AlereLAM is not designed to detect drug resistance; thus, it remains important to collect sputum to evaluate if there is rifampicin resistance, particularly in countries with a high prevalence of multidrug-resistant TB. It is crucial to educate patients about the importance of providing sputum samples even after AlereLAM has been done, and civil society organizations can assist health workers in creating awareness about the advantages and limitations of this test.

Substantial progress has been made in generating evidence for global policymaking and country evaluations that have provided insights into the placement of LF-LAM. This progress has been reflected not only in the WHO recommendations that were further expanded in 2019 and subsequent diagnostic algorithms (43) but also in the inclusion of AlereLAM as one of the elements to support decision-making when providing care for HIV-positive people with advanced disease (38), as well as its addition to the Global Drug Facility's TB Diagnostics Catalogue (47) and the First WHO Model List of Essential In Vitro Diagnostics (48). However, despite these policy changes, as well as the role of advocacy groups in increasing awareness and promotion of the test (49, 50), LF-LAM is yet to be fully adopted and implemented at the country level. The slow uptake of AlereLAM in low- and middle-income countries has been attributed to multiple factors, including delays in coordination between TB and HIV programmes (51, 52). While country programmes address existing challenges and as the TB diagnostic pipeline evolves, the rapid introduction of AlereLAM will serve not only as a means of reducing mortality associated with TB in HIV-positive people but also will facilitate uptake and widespread scale up of novel LAM-based tests as well as other point of care tools on the horizon.

QUALITY ASSURANCE

Quality assurance systems

The implementation of a comprehensive quality assurance system is essential to assure the accuracy, reliability and timeliness of any diagnostic test result for patients and programmes. Establishing a quality assurance system ensures that testing is completed according to national policies and regulations, appropriate samples of sufficient volume are received for testing, health workers are able to provide services that are not interrupted by stock-outs or defective tests or equipment, test results are returned to patients and clinicians in a timely manner, and the testing network is monitored and evaluated through measurement of its impact and, thus, is continually optimized. The essential elements of a quality assurance system for LF-LAM (Fig. 8) include developing and standardizing policies and documents, maintaining and servicing equipment (i.e. pipettors and centrifuges), providing initial and follow-up training for laboratory technicians and clinicians, implementing proficiency testing, strengthening the supply chain and monitoring quality indicators.

Fig. 8. Essential elements of a quality assurance system



For more information about these essential elements, including the need for appropriate LF-LAM policies and documents see [Implementation considerations](#), for training considerations see [Training staff](#), and for supply chain management and procurement see [Sample collection and storage](#) and [Procurement information](#). The remaining essential elements are discussed below.

Equipment service and maintenance

Testing sites that utilize electronic, automatic or calibrated pipettors or centrifuges, or a combination of these, to process frozen urine samples should ensure that devices are regularly recalibrated according to the manufacturer's instructions to ensure accurate and reliable results from LF-LAM. All maintenance activities should be undertaken by authorized technicians, and documentation should be kept at the testing facility for reference and to support a robust quality management system.

On-site supervisory visits

Regular staff training and supervisory visits should be conducted to determine the testing site's performance, provide testers with refresher training and identify any site-level needs not captured via routine reporting practices. Programmes should develop new or modify existing supervisory checklists to ensure a standardized tool is used during supervisory visits across the network so that findings can be compared over time and between testing sites. At sites already conducting other TB diagnostic tests, the use of LF-LAM can be evaluated and reports incorporated into the existing schedule for supervisory visits. At new testing facilities, particularly at point-of-care clinical sites, countries may need to designate and train supervisory staff to conduct site visits.

Quality control testing

Comprehensive quality control testing includes evaluating results from both internal and external control tests.

Internal quality control testing

Internal quality controls are used to demonstrate that a test is functioning properly and can produce a valid result. Each AlereLAM test strip includes an internal quality control window and result bar (Fig. 5) that should be evaluated for each test, as described in the next section and discussed in the [Reading and interpretation](#), as well as [Annex 2](#).

External quality control testing

While internal controls are used to assess the functionality of the test, external controls additionally evaluate the accuracy and reliability of the procedure by requiring staff to evaluate a known sample and achieve an acceptable result. While no product is currently designated for use as an external quality control sample for AlereLAM, countries may utilize known TB-negative and TB-positive urine samples with confirmed bacteriological results that have been retained under the recommended storage conditions for this purpose (see [Sample collection and storage](#)).

External quality control should be conducted centrally each time a new lot of AlereLAM test kits is received, prior to distribution of the strips to testing sites. New-lot testing ensures that manufacturing, shipping and storage conditions, such as temperature and humidity, have not negatively impacted the new lot's ability to produce an accurate result. New-lot AlereLAM test strips should be randomly selected for testing using both positive and negative external quality control samples. Results should be compared with results from the current or previous lot, and results should be documented in a new-lot testing logbook. New lots that do not achieve the expected results should not be distributed or used for patient testing.

In addition to new-lot testing, external quality control should be carried out weekly at each testing site before testing the first specimen is analysed during that particular week. If no specimens are to be tested by AlereLAM during a particular week, then quality control testing does not need to be undertaken. All quality control results should be recorded in an AlereLAM result logbook.

Use the following procedures to evaluate results from internal and external AlereLAM quality control samples.

AlereLAM positive control

- Label the test strip as the AlereLAM positive control.
- Add 60 µL of the AlereLAM positive control sample to the labelled test strip.
- Read the results after 25 minutes.
- Ensure that the quality control bar appears in the quality control window.
 - If the bar is present, the control result is acceptable. If a patient's sample was tested, the presence or absence of the patient bar should be observed in the patient window on the test strip, and the result should be interpreted and reported.
 - If the bar is not present, the sample should be repeated using a new test. If the bar does not appear on the repeat test strip, contact your local distributor or call Alere (Abbott) technical support.

AlereLAM negative control

- Label the test strip as the AlereLAM negative control.
- Add 2 drops of saline or distilled water to the labelled test strip.
- Read results after 10 minutes.
- Ensure that the quality control bar does not appear in the quality control window.
 - If the bar is not present, the control result is acceptable.
 - If the bar is present, the test should be repeated with a new negative control sample. If the bar appears on the repeat test, contact your local distributor or call Alere (Abbott) technical support.

Proficiency testing

Proficiency testing requires testers to process, test and report results for blinded, well-characterized samples for evaluation. Proficiency testing evaluates the accuracy and timeliness of prediagnostic (i.e. sample receipt and preparation), diagnostic (i.e. test functionality and tester proficiency) and postdiagnostic (i.e. result interpretation and reporting) processes and, therefore, serves as an efficient tool for monitoring and evaluation of testing networks. Proficiency testing programmes for LF-LAM are in development; however, countries should monitor the availability of these programmes to support their prompt implementation.

Monitoring and evaluation of quality indicators

As with all new diagnostic tests, countries should develop new or adapt existing quality standards and matched performance indicators for AlereLAM. Performance of AlereLAM at the site and network levels should be regularly evaluated using routinely collected and reported data (i.e. collated findings from supervisory visits, quarterly reports or other existing channels of documentation). Unexpected changes in performance or performance that is below targets for quality should be promptly investigated for remediation. Examples of quality standards and performance indicators can be found in the GII training package for diagnostic network strengthening (53).

PROCUREMENT INFORMATION

Because AlereLAM is a rapid strip-based test, procurement is relatively simple compared with other TB diagnostics that employ multiple reagents and consumables with varying shelf-life and storage requirements. However, because the test may be used at the point of care, its widespread deployment in a country requires careful supply chain management to avoid stock-outs and to ensure that tests are used before their expiry dates, especially in peripheral settings. This section provides information about the supplies and accessories needed for the test, as well as guidance for forecasting order sizes.

Before submitting an order, countries interested in introducing AlereLAM should contact the manufacturer about their intention to procure the test to ensure that appropriate regulatory procedures are initiated. It should be anticipated that the manufacturer will need time to develop and submit documentation and provide any required supplementary information.

Product information

The basic product information is:

- product name – Alere Determine™ TB LAM Ag assay;
- manufacturer – Abbott Laboratories (formerly Alere Inc.);
- cost – US\$ 3.50/test strip (packaged in kits of 100 test strips: US\$ 350);
- Global Drug Facility (GDF) item number – 106642 (<http://stoptb.org/assets/documents/gdf/drugsupply/GDFDiagnosticsCatalog.pdf>); to place an order through GDF, contact gdf@stoptb.org;
- shelf life – 18 months;
- storage conditions – 2–30 °C.

Table 1 outlines the equipment necessary to perform AlereLAM in addition to that provided in the kit.

Table 1. Accessories required but not provided in the Alere Determine TB LAM Ag assay kit

Equipment needed	Global Drug Facility catalogue description and product code number	Units per pack	Cost in catalogue (2020)
Urine collection cups	Specimen collection cups, 80 mL each GDF product code: 106525	1 000	US\$ 83.30
Pipette capable of delivering 60 µL	Pipette capable of delivering 10–100 µL GDF product code: 106055	1	US\$ 226.94
Disposable pipette tips	Pipette tips capable of delivering 10–100 µL (1 000 tips/pack) GDF product code: 106388	10 × 96	US\$ 72.75
Dual-bulb micropipette ^a	Dual-bulb Pasteur pipettes with volume of 60 µL for exact transfer of sample Non-graduated, non-sterile pipettes can be used	Not available	Not yet available. Prices will be published in the catalogue.
Timer	Mechanical timer GDF product code: 106570	1	US\$ 1.11

GDF: Global Drug Facility.

^a Expected to be included in the GDF catalogue. As an alternative to a pipette and tips, and to facilitate use of the test in peripheral settings, disposable dual-bulb micropipettes may be used. However, the accuracy of the dual-bulb pipettes should be tested against a calibrated pipette before they are put into widespread use.

Forecasting orders for rolling out AlereLAM

AlereLAM test kits have a shelf-life of 18 months; thus, ordering a 1-year supply allows for a buffer in case implementation progresses more slowly than expected. Splitting a 1-year order into two deliveries allows for an even greater buffer; the second planned delivery date may be postponed if required. (The information in this section is taken from the GDF technical information note at http://www.stoptb.org/assets/documents/gdf/GDF_Technical_Information_Note_DetermineTM_TB_LAM_Ag_test_180604-SPREADS.pdf).

Regardless of the algorithm used, clinicians must be properly trained to prevent an underuse of tests and the consequent expiration and wastage of tests or, conversely, the inappropriate overuse and early depletion of stocks.

The steps below should aid in estimating how many tests are needed for a 1-year supply, based on full adoption of these recommendations.

- The number of tests ordered should equal the number of HIV-positive people who were tested for TB in the previous year at the implementation sites (i.e. those with signs or symptoms, who have AHD or who were seriously ill), based on a review of registers. If CD4 counts are routinely measured at sites, there will be additional patients without signs or symptoms of TB but who should be tested with LF-LAM based on the CD4 count criteria specified in the revised recommendations for inpatient and outpatient settings; including these patients may justify an approximately 20% increase in the order size, given that most patients with low CD4 counts would be expected to have a sign or symptom of TB during a given year.
- In the absence of data from registers about the number of HIV-positive people who were tested for TB in the previous year, 0.3 (30%) may be used as a default estimate of the proportion of HIV-positive people who will be tested for TB in a given year.³
- For subsequent forecasting as the country moves towards nationwide implementation of the 2019 recommendations, the registered number of people living with HIV nationwide who were tested for TB during the previous year should be considered the target to phase in over time. Data that become available about actual test consumption rates and speed of uptake at new sites should be used to refine the forecasts and order plans.

LF-LAM rapid diagnostic tests in the pipeline

In September 2018, the Foundation for Innovative New Diagnostics (FIND) announced that a novel urine-based assay had been co-developed with Fujifilm (Tokyo, Japan). The test is known as the Fujifilm SILVAMP TB-LAM, or simply FujilAM; it is a rapid diagnostic test able to detect low concentrations of LAM antigen in people with TB–HIV coinfection (54). In 2019, a call for partner trials was opened for the prospective evaluation of this test. In addition to the initial FIND trial, which defined the sensitivity and specificity of the test using biobanked urine samples, other trials are under way to evaluate the test's accuracy, performance and feasibility under programmatic conditions. However, research studies are still being conducted and the test is not commercially available. Further, other initiatives are under way within the global research and development community to design the best TB LAM rapid diagnostic test, ideally one that will move beyond the current restricted use and provide a rapid LF assay to diagnose TB regardless of HIV status, across all ages, and for all forms of active TB disease.

For now, implementation of AlereLAM should be strongly promoted, as evidence demonstrates that the use of this test improves the management of HIV-positive people (i.e. those with AHD, low CD4 cell counts or who are seriously ill) and increases TB detection in this vulnerable population when used in conjunction with other tests.

³ WHO proposes a default factor of 0.3 to estimate the proportion of all HIV-positive people who would have signs or symptoms of TB in a given year, assuming patients are clinically screened on average twice a year and 15% of screened patients would have signs or symptoms (55).

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ANNEXES

ANNEX 1. SUMMARY OF POLICY CHANGES, 2015 AND 2019

Table A1.1. Changes in evidence-based recommendations on the use of the lateral flow lipoarabinomannan assay (LF-LAM)^a

<i>The use of lateral flow urine lipoarabinomannan assay (LF-LAM) for the diagnosis and screening of active tuberculosis in people living with HIV: policy guidance (2015)^b</i>	<i>Lateral flow urine lipoarabinomannan assay (LF-LAM) for the diagnosis of active tuberculosis in people living with HIV: policy update 2019^c</i>	Changes from 2015 to 2019
<p>In inpatient settings, LF-LAM may be used to assist in diagnosing TB in HIV-positive adult patients:</p> <ul style="list-style-type: none"> • who have signs and symptoms of TB (pulmonary or extrapulmonary, or both); OR • in those who have a CD4 cell count ≤ 100 cells/ mm³; OR • in HIV-positive patients who are seriously ill, regardless of their CD4 cell count or if their CD4 cell count is unknown. 	<p>In inpatient settings, WHO strongly recommends using LF-LAM to assist in diagnosing active TB in all HIV-positive adults, adolescents and children:</p> <ul style="list-style-type: none"> • who have signs and symptoms of TB (pulmonary or extrapulmonary, or both); OR • who have advanced HIV disease or are seriously ill; OR • have a CD4 cell count < 200 cells/ mm³ irrespective of whether there are signs and symptoms of TB. 	<p>Increased strength of the recommendation.</p> <p>Improved quality of evidence.</p> <p>Increased scope of the recommendation to include:</p> <ul style="list-style-type: none"> • all symptomatic or seriously ill inpatients, irrespective of their CD4 cell count; • all inpatients with advanced HIV disease; and • inpatients with or without signs and symptoms of TB who have a CD4 cell count < 200 cells/ mm³.
<p>The use of LF-LAM also applies to HIV-positive adult outpatients who have signs and symptoms of TB (pulmonary or extrapulmonary) who:</p> <ul style="list-style-type: none"> • have a CD4 cell count ≤ 100 cells/mm³; OR • are seriously ill, regardless of their CD4 cell count or if their CD4 cell count is unknown. 	<p>In outpatient settings, WHO suggests using LF-LAM to assist in diagnosing active TB in all HIV-positive adults, adolescents and children who:</p> <ul style="list-style-type: none"> • have signs and symptoms of TB (pulmonary or extrapulmonary, or both) OR who are seriously ill: OR • have a CD4 cell count < 100 cells/ mm³ irrespective of whether there are signs and symptoms of TB. 	<p>Increased scope of the recommendation to include:</p> <ul style="list-style-type: none"> • all outpatients with signs and symptoms of TB OR who are seriously ill; AND • all outpatients who have a CD4 cell count < 100 cells/ mm³irrespective of whether there are signs and symptoms of TB.

<p>Except as specifically described above for HIV-positive people (i.e. low CD4 cell counts or who are seriously ill), LF-LAM should not be used to diagnose TB.</p>	<p>In outpatient settings, WHO recommends against using LF-LAM to assist in diagnosing active TB in HIV-positive adults, adolescents and children:</p> <ul style="list-style-type: none"> • without assessing TB symptoms; • who do not have TB symptoms and whose CD4 cell count is unknown OR who do not have TB symptoms and have a CD4 cell count > 200 cells/ mm³; OR • who do not have TB symptoms and who have a CD4 cell count of 100–200 cells/mm³: 	<p>Patient populations are better defined for the negative recommendation against using LF-LAM.</p>
<p>LF-LAM should not be used as a screening test for TB.</p>	<p>See the recommendations above for inpatients and outpatients for situations in which LF-LAM is suggested for use among HIV-positive individuals, irrespective of whether they have signs and symptoms of TB.</p> <p>See the recommendations above for outpatients for situations in which WHO recommends against using LF-LAM testing.</p>	<p>Clarification of recommendation for using LF-LAM among HIV-positive individuals with and without signs and symptoms of TB:</p> <ul style="list-style-type: none"> • LF-LAM is strongly recommended for HIV-positive inpatients with advanced HIV disease and individuals with a CD4 cell count < 200 cells/mm³, irrespective of whether there are symptoms of TB; AND • LF-LAM testing is suggested for use for HIV-positive outpatients with a CD4 cell count < 100 cells/ mm³ irrespective of whether there are signs and symptoms of TB.
<p>The initial recommendation also applies to HIV-positive children with signs and symptoms of TB (pulmonary or extrapulmonary) and was based on the generalization of data from adults, with the acknowledgment that the data were limited and there was concern regarding the low specificity of LF-LAM in children.</p>	<p>The new recommendations also apply to HIV-positive adolescents and children and are based on the generalization of data from adults with the acknowledgment that data for these population groups are limited.</p>	

^a This table summarizes the guidance in WHO's 2015 and 2019 publications. Additional information, such as the strength of the recommendations, can be found in the guidance.

^b The use of lateral flow urine lipoarabinomannan assay (LF-LAM) for the diagnosis and screening of active tuberculosis in people living with HIV: policy guidance. Geneva: World Health Organization; 2015 (WHO/HTM/TB/2015.25; <https://apps.who.int/iris/handle/10665/193633>).

^c Lateral flow urine lipoarabinomannan assay (LF-LAM) for the diagnosis of active tuberculosis in people living with HIV: policy update 019. Geneva: World Health Organization; 2019 (WHO/CDS/TB/2019.16; <https://apps.who.int/iris/handle/10665/329479>).

ANNEX 2. STANDARD OPERATING PROCEDURE FOR PERFORMING THE ALERE DETERMINE TB LAM AG LATERAL FLOW ASSAY

Purpose

The purpose of this standard operating procedure⁴ is to detail the steps for correctly performing, interpreting and documenting valid results for the Alere Determine™ TB LAM Ag assay (AlereLam). AlereLAM is an immunoassay used to detect the lipoarabinomannan (LAM) antigen (Ag) in human urine as an aid in diagnosing TB in persons living with HIV.

Scope

This standard operating procedure applies to all facilities performing AlereLAM to assist in diagnosing TB in HIV-positive adults who have signs and symptoms of TB (pulmonary or extrapulmonary) and who have a CD4 count < 100cells/mm³ or who are seriously ill (WHO stage 3 or 4 disease).

Responsibility and authorization

The persons responsible for performing this test are laboratory technologists and trained non- laboratory personnel (e.g. nurses, people providing HIV testing and counselling).

Materials

- AlereLAM test kit and Reference Scale Card
 - AlereLAM antigen test strips
 - AlereLAM positive TB control sample (1 mL).
- Materials required but not provided in the kit
 - Timer
 - Gloves
 - Pipette or delivery device capable of accurately delivering 60 µL of urine (this could be a calibrated micropipette with filter tips or a dual-bulb 60 µL pipette)
 - Pipette filter tips if a micropipette is used
 - Sharps disposal container
 - Pen and permanent marker
 - Biohazard disposal bags.

Safety, health and the environment

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

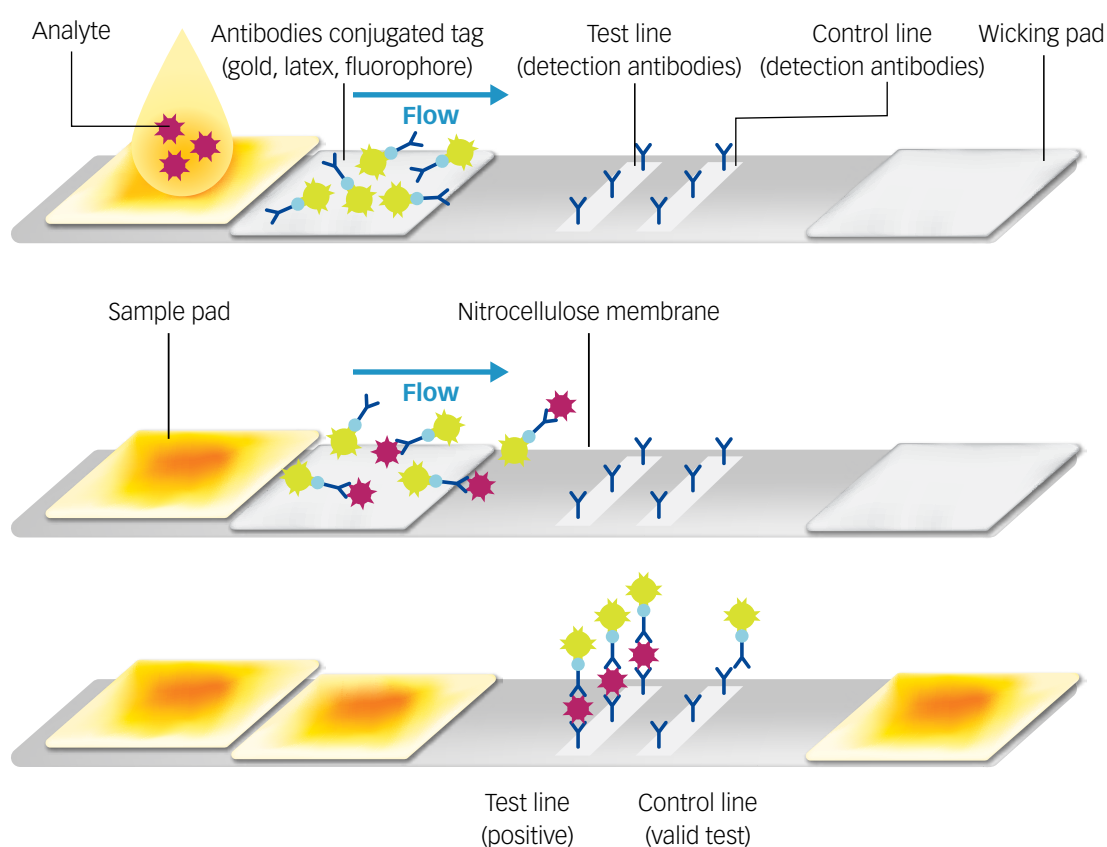
⁴ This standard operating procedure was adapted from Médecins Sans Frontières.

Principles

AlereLAM is an immunochromatographic test for the qualitative detection of LAM antigen in human urine. AlereLAM employs highly purified antibodies specific to the major polysaccharide antigen of *Mycobacterium*: 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 through conjugation to colloidal gold particles (Fig. A2.1).

After a urine specimen is added to the test strip, the colloidal gold–conjugated antibodies attach to the LAM antigen and are released by the specimen from the test strip. 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 purple–grey band) indicates that LAM antigen is present in the sample at or above the detection limit of the test; a negative result (no purple–grey line) indicates it is not present or is present only below the detection limit. To ensure assay validity, a procedural control window is incorporated into the assay device.

Fig. A2.1. General principles* for detecting the lipoarabinomannan antigen



* Sample containing the analyte of interest moves by capillary action across an internal membrane when applied to the assay where it will bind first to capture antibodies which have a reporter molecule attached. The analyte-antibody complex then continues to migrate until reaching another set of detection antibodies fixed to the membrane which binds the complexed molecules, concentrating them in one place (test line) for detection. Any remaining unbound capture antibodies continue to migrate and complex to a second set of fixed antibodies at a control line which validates the test.

Specimen collection and storage

Collect midstream urine in a clean, standard urine collection container. Fresh urine samples can be used within 8 hours if kept at room temperature.

- (1) Urine samples should be stored at 2–8 °C if the test is to be run within 3 days of collection.
- (2) If testing will be delayed more than 3 days, the samples should be frozen (–20 °C or colder). For frozen or refrigerated urine, bring the sample to room temperature 1 hour prior to use. Frozen samples may contain aggregates.
- (3) All thawed samples must be centrifuged at 10 000 g for 5 minutes at room temperature; 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 three times cannot be used.

Reagent storage and preparation

AlereLAM test cards must be stored at 2–30 °C until they are used. Kit components are stable until the expiration date when handled and stored as directed. Do not use kit components beyond the expiration date. Immediately reseal all unused tests in the foil pouch containing the desiccant by pressing the seal from end to end to close. Do not use strips that have become wet, and do not use strips if the packaging has become damaged.

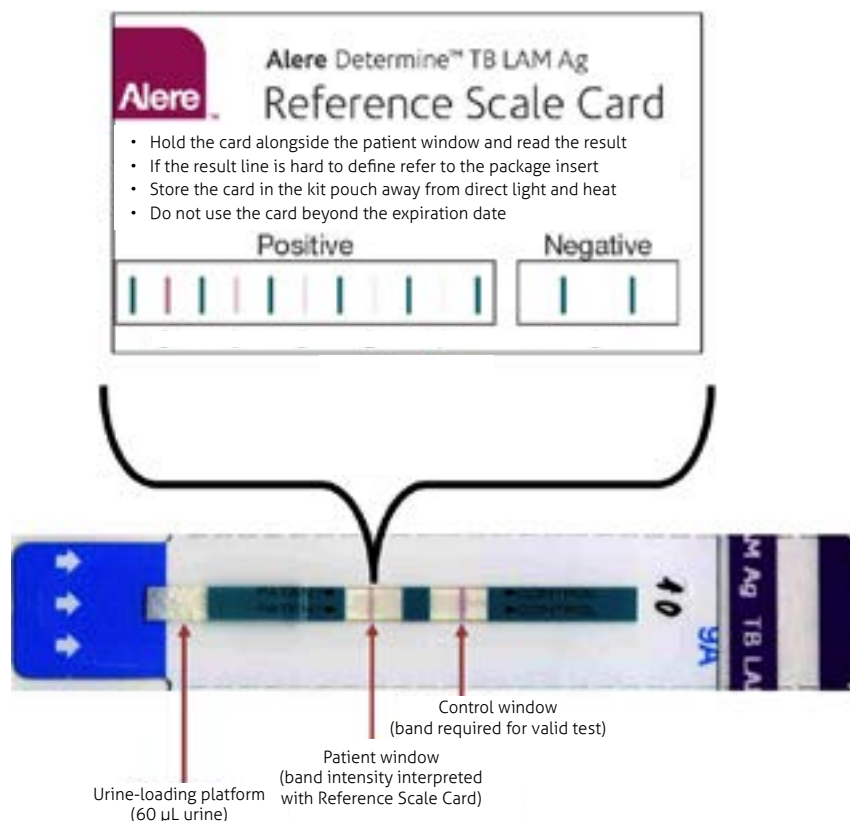
Test procedure

- (1) Remove the desired number of test strips from the 10-test card by bending and tearing at the perforation. Test strips should be removed starting from the right side of the test card to preserve the lot number, which appears on the left side of the card.
- (2) Remove the protective foil cover from each test strip. Label the strip with a unique patient identification number. The assay should be initiated within 2 hours of removing the protective foil cover from the strip.
- (3) Add 60 µL of the sample (or 2 drops of urine) to the test strip (Fig. A2.1; the white pad marked with an arrow symbol).
- (4) Wait a minimum of 25 minutes and a maximum of 35 minutes, and then read the result. Evaluate the strip under standard indoor lighting conditions or in the shade. Do not evaluate the strip in direct sunlight. Results are stable for up to 35 minutes after sample application. Do not read the strip after 35 minutes.

Interpreting the results

To assist with reading and interpreting the results, use the Reference Scale Card that is provided in the kit by holding it alongside the patient window (Fig. A2.2).

Fig. A2.2. Using the Reference Scale Card to determine band intensity and validity of the Alere Determine TB LAM Ag assay



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LAM antigen positive result (showing two bands, the control and patient band)

If a test is positive, then purple–grey bands appear in both the quality control window and the patient window of the strip. Note that the test result is positive even if the patient band appears lighter or darker than the control band.

Note: The reference card must be used to correctly identify the intensity of the bands appearing in the patient window. Bands that are lighter than the bands in the positive box of the reference card must be considered as negative or indeterminate results. Only bands that are as dark or darker than the first band in the positive box of the reference card should be considered positive.

Negative result (only one band showing)

The result is negative if a purple–grey band appears only in the quality control window of the strip and no band or only a band of Grade 1 intensity appears in the patient window.

Invalid result (no band)

The test is invalid if there is no purple–grey band in the quality control window of the strip, even if a band appears in the patient window; in this case, the test should be repeated. If the problem persists, contact your local distributor or call Alere Technical Support.

Indeterminate result

The result is indeterminate if one purple–grey band appears in the control window of the strip with an unclear or incomplete band in the patient window. To ensure that a better clinical decision is made, the test should be repeated. Alternatively, collect a new urine sample from the patient on a different day and test that sample. Early morning urine is recommended.

Quality control testing

Conduct quality control testing for AlereLAM weekly, before the first specimen is analysed for a particular week. If no specimens are to be test using the AlereLAM assay, then quality control testing need not be undertaken for that week. Record the results of the quality control testing in the TB LAM result logbook.

The following procedure should be used to evaluate the AlereLAM quality controls.

For the AlereLAM positive control:

- (1) first, label the test strip as the TB LAM positive control;
- (2) add 1 drop of the TB LAM Ag positive control to the labelled test strip;
- (3) read the results after 25 minutes.

For the AlereLAM negative control:

- (1) first, label the test strip as the TB LAM negative control;
- (2) add 2 drops of saline solution or distilled water;
- (3) read the results after 10 minutes.

Further information can be found on the Alere Determine LAM Ag package insert, available at <https://www.alere.com/en/home/product-details/determine-tb-lam.html>.

