Introduction to the GLI guide for the practical implementation of lateral flow urine lipoarabinomannan assay (LF-LAM)

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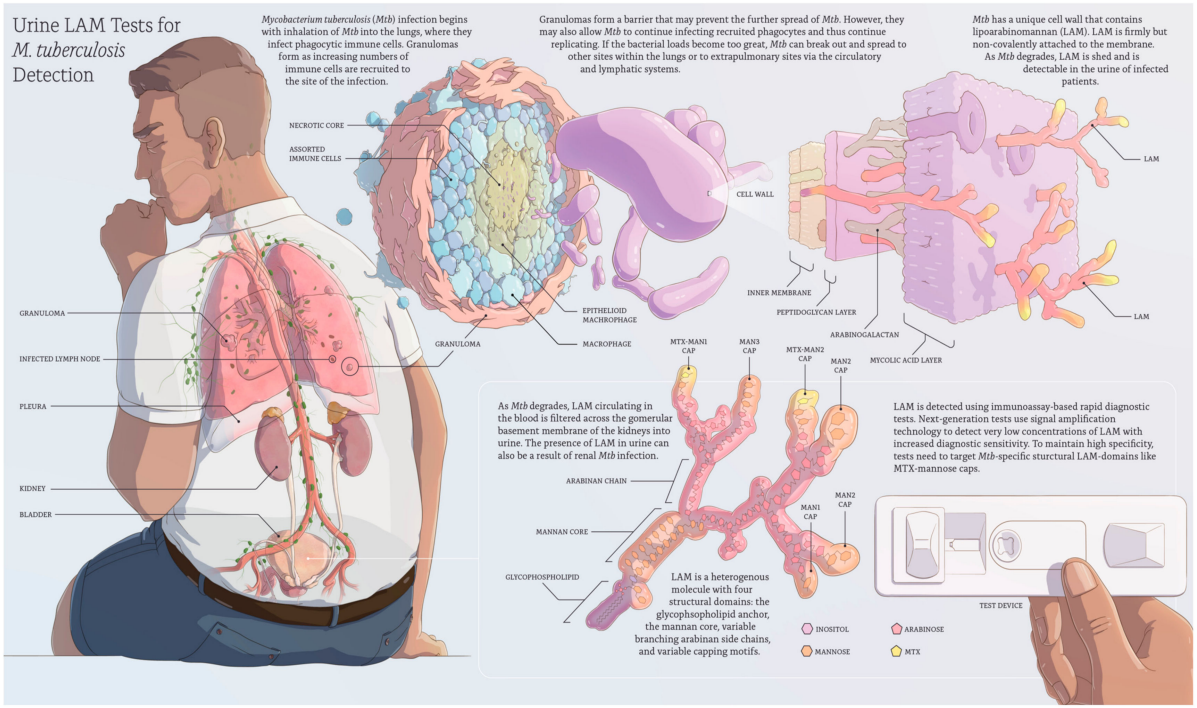
LF-LAM: an introduction



- Lipoarabinomannan (LAM) is a mycobacterial cell wall lipopolysaccharide
- LAM encompasses a large family of related molecules which are expressed by mycobacterial species
- LAM in urine:
  - -LAM circulating in the blood is filtered by glomerular basement membrane
  - -Dissemination to the kidneys
    Marker for disseminated TB

Bulterys et al. J. Clin. Med. 2020, 9, 111

LF-LAM as a test for TB diagnosis in PLHIV: key features (1)

	Abbott Determine TB LAM Ag (AlereLAM)		Sputum-Based TB Diagnosis (Smear Microscopy; Xpert MTB/RIF; Xpert MTB/RIF Ultra)	
Price per test (ex-works)	✓	US$3.00–3.50 [17] (likely cost-effective among hospitalized HIV-postive patients)	x	Xpert MTB/RIF US$9.98 [19]
Regulatory requirements and availability	✓	CE-IVD marked IVD WHO recommendation (Table 2) On the market	✓	CE-IVD marked IVD Several WHO recommendations On the market
Equipment	✓	Instrument free	x	US$17,500.00 [19] (GeneXpert platform plus laptop)
Sensitivity in HIV positive patients (independent of CD4 count)	x	42% [21]	✓	90% (Xpert MTB/RIF Ultra) [23] 77% (Xpert MTB/RIF) [23] 47% (Microscopy) [24]
Sensitivity in HIV negative patients	x	18% [25]	✓	91% (Xpert MTB/RIF Ultra) [23] 90% (Xpert MTB/RIF) [23]
Specificity	?	96–98% against CRS [26] (likely meeting the target as specificity might be underestimated due to limitations of the reference standard)	✓	96% (Xpert MTB/RIF Ultra) [23] 98% (Xpert MTB/RIF) [23] 98% (Microscopy) [24]
Day 1 diagnostic yield in HIV-positive inpatients (TB patients diagnosed on the first day they present)		43.3% [27]		26.2% (Xpert MTB/RIF) [27] 19.1% (Microscopy) [27]
Outcome/ / mortality impact		Mortality impact shown in hospitalized PLHIV but not in more general populations. A positive result is associated with increased risk of mortality [28–30]		Unclear
Sample type	✓	Urine	x	Sputum
Time-to-result	✓	25 min	x	100 min (Xpert MTB/RIF and Xpert MTB/RIF Ultra)
Number of steps	✓	2 steps	x	Xpert MTB/RIF and Xpert MTB/RIF Ultra: 11 steps Microscopy: >10 steps
Setting and infrastructure needs	✓	Simple to use lateral flow assay	x	Laboratory required Electricity required Equipment susceptible to dust and shock

Table adapted from Bulterys et al. J. Clin. Med. 2020, 9, 111

❑ Alere™ Determine TB LAM Ag test

- Lateral flow assay, only truly POC test currently available for TB diagnosis
- Based on urine: sample easy to collect; minimal biosafety requirements
- Rapid test (25 minutes)-can support same-day diagnosis
- Sensitivity in symptomatic **PLHIV** (all settings): 42%
  (Bjerrum et al., Cochrane systematic review 2019)
- Decrease on 8-weeks mortality in symptomatic PLHIV in inpatient settings (Peters et al., 2016)

LF-LAM as a test for TB diagnosis in PLHIV: key features (2)

❑ Why is LF-LAM a useful addition to TB diagnostic algorithm ?

- Addition of urine LF-LAM to the diagnostic algorithms for management of either hospitalized or ambulatory HIV + people significantly increases the diagnostic yield

- Although less significant, an increase in detection yield has also been observed when LF-LAM is added to a diagnostic algorithm that includes Xpert MTB/RIF.
  LF-LAM has an added value :
  - **for patients who can not produce sputum**
  - **when Xpert is not available on site**

- LF-LAM **can support same day diagnosis**. Critical for PLHIV who are at higher risk of mortality yet unable to produce adequate samples for diagnostic evaluation

Peters et al 2016; Zijnah et al 2016 ; Huerga et al 2017; Sabur et al 2017; Huerga et al 2020; Sossen et al 2021

Symptomatic PLHIV: stratification by settings

Patient population	Pooled Sensitivity*	Pooled Specificity*
All settings	42%	91%
Inpatients	52%	87%
Outpatients	29%	96%

*pooled sensitivity increased, and specificity decreased with lower CD4 cell count
Source: Bjerrum et al., Cochrane systematic review 2019

Unselected PLHIV : stratification by settings

Patient population	Pooled Sensitivity*	Pooled Specificity*
All settings	35%	95%
Inpatients	62%	84%
Outpatients	31%	95%

*pooled sensitivity increased, and specificity decreased with lower CD4 cell count
Source: Bjerrum et al., Cochrane systematic review 2019

LF-LAM: an overview of policies and guidance

- **WHO 2019 LF-LAM policy update**
  - Increased strength of recommendation
  - Improved quality of evidence
  - Increased scope of recommendations (revised target population eligible for LF-LAM testing)

- **WHO Operational Handbook on TB, Module 3: Diagnosis**
  - Updated LF-LAM algorithms

LF-LAM: GLI guide

❑ GLI practical guide for LF-LAM

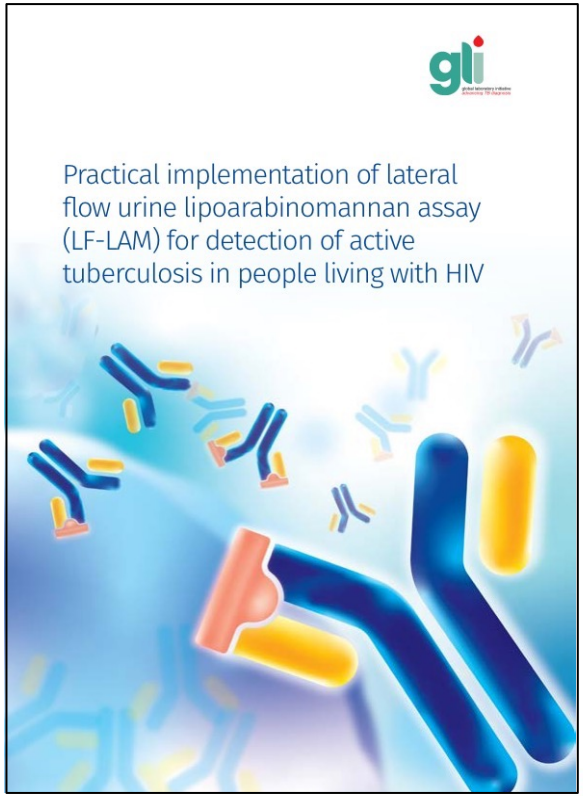
- Focus on commercially available tests (Alere™ Determine TB LAM Ag test)

TARGET AUDIENCE:

- National TB and HIV programs, front-line HCWs, implementers

CONTENTS:

- Basics (i.e. assay principles, sample collection, procedures, interpretation etc)
- Role of LF-LAM in TB diagnostic algorithm
- Practical considerations for country introduction and roll-out
- Quality assurance
- Procurement Information
- Laboratory SOP (Annex)



GLI guide and role of LF-LAM for TB diagnosis: latest WHO policy recommendations

Inpatient settings

- **WHO strongly recommends using LF-LAM 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), **irrespective of CD4 count**

- **irrespective of signs and symptoms of TB**
  - with advanced HIV disease (AHD) or seriously ill
  - with **a CD4 count ≤ 200 cells/mm^3**

Outpatient settings

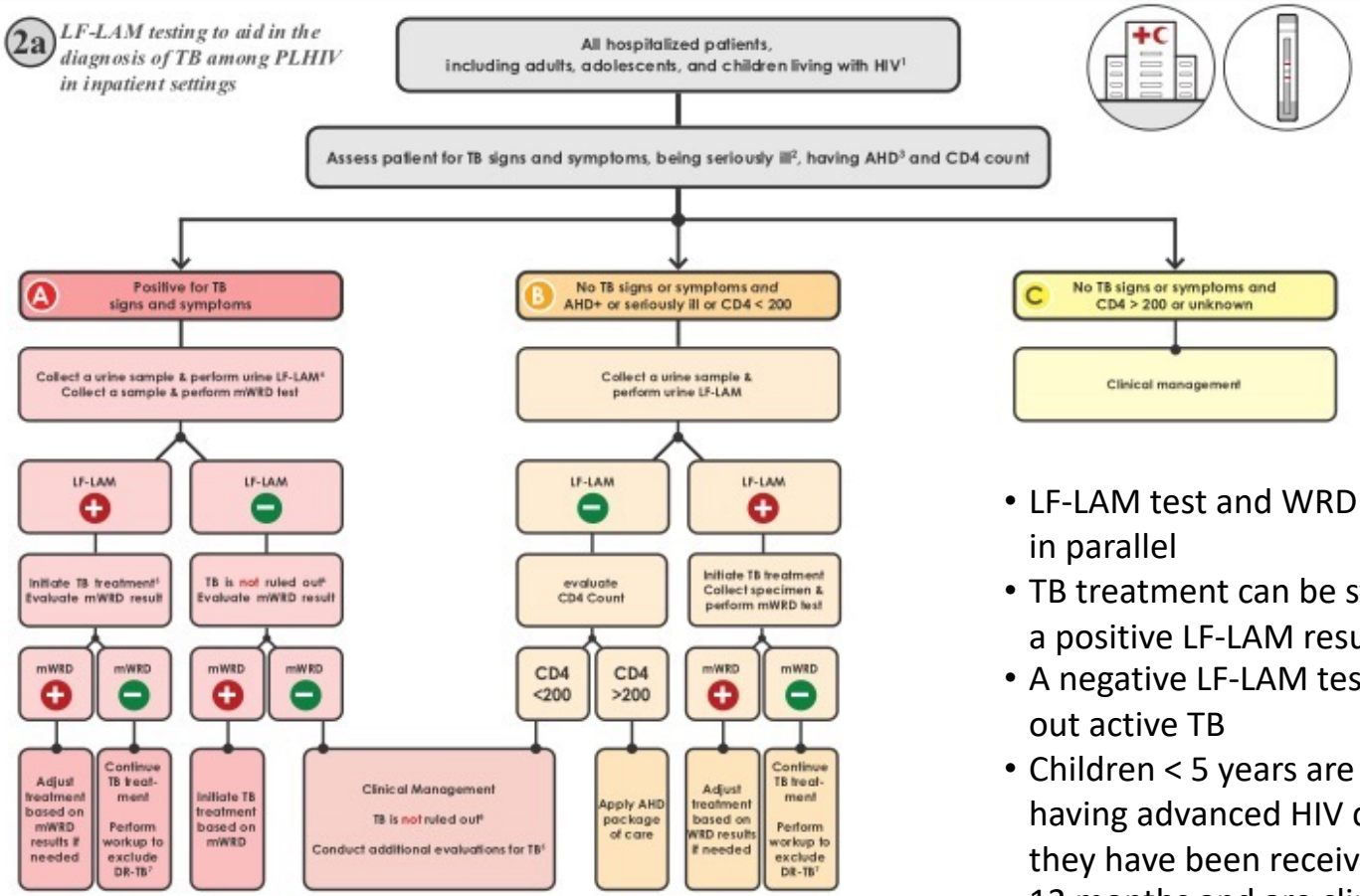
- **WHO suggests using LF-LAM to assist in the diagnosis of active TB in HIV-positive adults, adolescents and children:**

- **with signs and symptoms of TB** (pulmonary and/or extrapulmonary) **and/or seriously ill, irrespective of CD4 count**

- **irrespective of signs and symptoms of TB** and **CD4 count of less than 100 cells/mm^3**

WHO 2019 policy update on LF-LAM (https://www.who.int/publications/i/item/9789241550604)

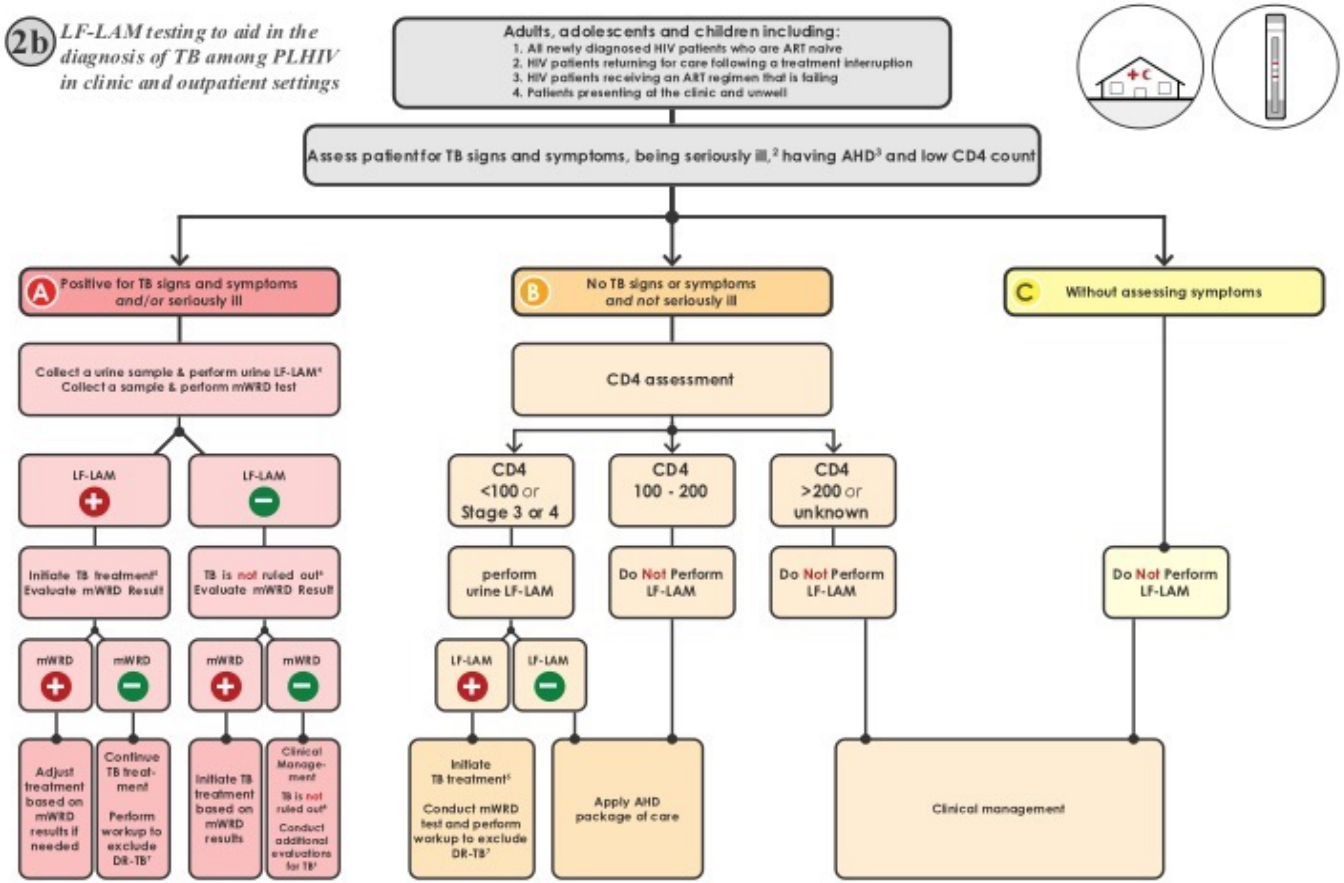
LF-LAM for TB diagnosis: Inpatient settings



- LF-LAM test and WRD should be done in parallel
- TB treatment can be started based on a positive LF-LAM result
- A negative LF-LAM test can NOT rule-out active TB
- Children < 5 years are considered as having advanced HIV disease, unless they have been receiving ARVs for > 12 months and are clinically stable*

WHO operational handbook on tuberculosis. Module 3: diagnosis - rapid diagnostics for tuberculosis detection (2020)
*Package of care for children and adolescents with advanced HIV disease. Technical brief. WHO, July 2020

LF-LAM for TB diagnosis: Outpatient settings



- PLHIV who are NOT seriously ill and do not have TB signs and symptoms, are eligible for LF-LAM testing only if CD4 count is < **100 cells/mm^3** or if they are Stage 3 or 4
- LF-LAM test and WRD should be done in parallel, whenever possible
- TB treatment can be started based on a positive LF-LAM result
- A negative LF-LAM test can NOT rule-out active TB
- Children < 5 years are considered as having advanced HIV disease, unless they have been receiving ARVs for more than 12 months and are clinically stable*

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GLI guide on LF-LAM: Practical considerations for roll-out

❑ Practical consideration for introduction and roll-out

- Kick-off procedure for in country registration
- **Establish a WG/platform gathering HIV and TB technical experts and national program representatives**
- **Define and agree on Target Population and placement of LF-LAM in health care settings (incorporation of 2019 WHO policy into national policies)**
- **Define placement in TB diagnostic algorithm**
- **Recording and reporting: LF-LAM positive results to be recorded and reported as bacteriologically confirmed***
- Develop procurement plans and forecast
- Develop training program and key training material
- Plan for phased introduction
- Roll-out training program
- Support /supervision and monitoring of consumption

*Definitions and reporting framework for tuberculosis – 2013 revision: updated December 2014 and January 2020. WHO 2020.

GLI practical guide on LF-LAM: Site-level implementation

❑ Based on experience from early implementers:

- **TRAINING NEEDS:**
  - Important to train HCWs on patients eligibility for LF-LAM testing
  - Adherence to diagnostic algorithm, especially for negative results and for testing of drug resistance
  - Test performance (1-4 h training)

- **LOCATION FOR TEST PERFORMANCE and TIME TO RESULT**
  - Feasible to perform test in consultation room
  - Same day result (average TAT < 2 hours)

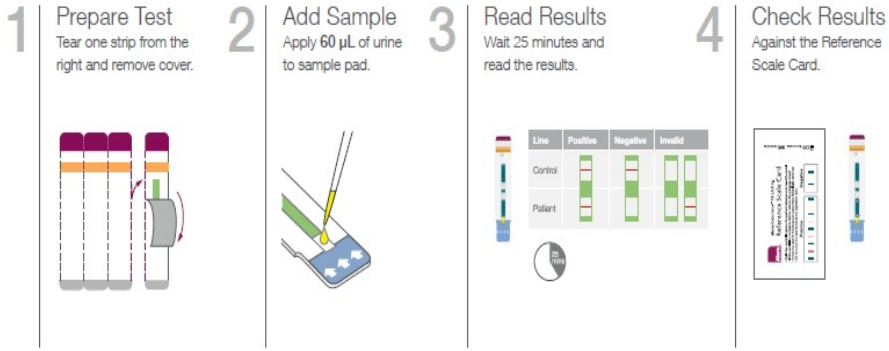
- **AVAILABILITY OF SANITARY SERVICES and DEDICATED PLACES TO SAFELY DISCARD SAMPLES**
  - Ensure privacy for sample collection
  - Ensure availability of places to safely dispose urine samples

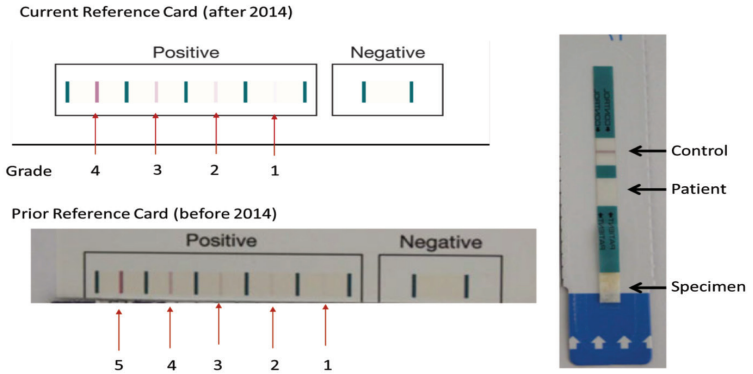
- **PATIENT FLOW**
  - If CD4 testing is needed: referral, additional visits, patient follow-up

GLI guide on LF-LAM: Test performance

- **SUPPLY NEEDED FOR PERFOMANCE OF TEST**
  - Including items not included in test kits
- **SAMPLE COLLECTION AND SAMPLE STORAGE**
  -Conditions and timelines for sample storage
- **PROCEDURES**
  - Detailed description of steps required
- **RESULTS READ-OUT and INTERPRETATION**
  - Band intensity and use reference card
- **BIOSAFETY and WASTE DISPOSAL**
  - Minimal biosafety requirements
  - Ensure adequate disposal of samples

Detailed SOP in the Annex





Source: WHO LF-LAM Policy Guidance (2015)

GLI practical guide on LF-LAM: Procurement

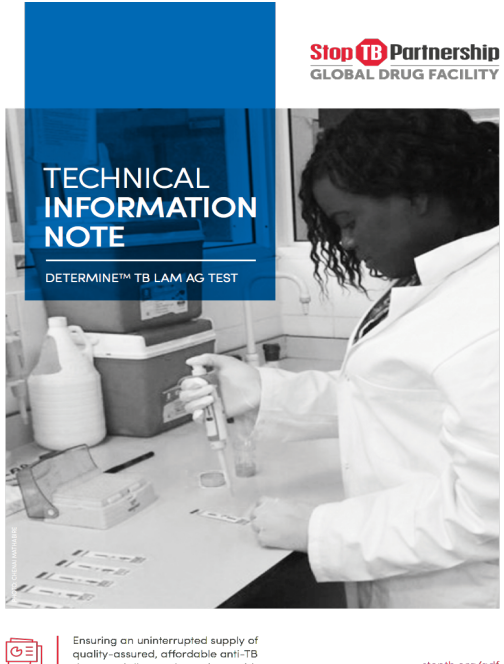
- **DETAILED LIST OF ITEMS NEEDED FOR PERFOMANCE OF TEST -** Availability through GDF
- **METHODOLOGY FOR QUANTIFICATION**

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.



gli global laboratory initiative advancing TB diagnosis

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