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

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ASLM-GLI Webinar
June 30, 2021
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
LF-LAM as a test for TB diagnosis in PLHIV: key features (1)

- **AlereTM 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)

<table>
<thead>
<tr>
<th>Abbott Determine TB LAM Ag (AlereLAM)</th>
<th>Sputum-Based TB Diagnosis (Smear Microscopy; Xpert MTB/RIF; Xpert MTB/RIF Ultra)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Regulatory requirements and availability</strong></td>
<td>✅ CE-IVD marked IVD, WHO recommendation (Table 2) On the market</td>
</tr>
<tr>
<td><strong>Equipment</strong></td>
<td>✅ Instrument free</td>
</tr>
<tr>
<td><strong>Sensitivity in HIV positive patients (independent of CD4 count)</strong></td>
<td>✗ 42% [21]</td>
</tr>
<tr>
<td><strong>Sensitivity in HIV negative patients</strong></td>
<td>✗ 18% [25]</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>✗ 96–98% against CRS [26] (likely meeting the target as specificity might be underestimated due to limitations of the reference standard)</td>
</tr>
<tr>
<td><strong>Day-1 diagnostic yield in HIV-positive inpatients (TB patients diagnosed on the first day they present)</strong></td>
<td>43.3% [27]</td>
</tr>
<tr>
<td><strong>Outcome/ mortality impact</strong></td>
<td>Mortality impact shown in hospitalized PLHIV but not in more general populations. A positive result is associated with increased risk of mortality [28–30]</td>
</tr>
<tr>
<td><strong>Sample type</strong></td>
<td>✅ Urine</td>
</tr>
<tr>
<td><strong>Time-to-result</strong></td>
<td>✅ 25 min</td>
</tr>
<tr>
<td><strong>Number of steps</strong></td>
<td>✅ 2 steps</td>
</tr>
<tr>
<td><strong>Setting and infrastructure needs</strong></td>
<td>✅ Simple to use lateral flow assay</td>
</tr>
</tbody>
</table>

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

### Symptomatic PLHIV: stratification by settings

<table>
<thead>
<tr>
<th>Patient population</th>
<th>Pooled Sensitivity*</th>
<th>Pooled Specificity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>All settings</td>
<td>42%</td>
<td>91%</td>
</tr>
<tr>
<td>Inpatients</td>
<td>52%</td>
<td>87%</td>
</tr>
<tr>
<td>Outpatients</td>
<td>29%</td>
<td>96%</td>
</tr>
</tbody>
</table>

*pooled sensitivity increased, and specificity decreased with lower CD4 cell count

Source: Bjerrum et al., Cochrane systematic review 2019

### Unselected PLHIV: stratification by settings

<table>
<thead>
<tr>
<th>Patient population</th>
<th>Pooled Sensitivity*</th>
<th>Pooled Specificity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>All settings</td>
<td>35%</td>
<td>95%</td>
</tr>
<tr>
<td>Inpatients</td>
<td>62%</td>
<td>84%</td>
</tr>
<tr>
<td>Outpatients</td>
<td>31%</td>
<td>95%</td>
</tr>
</tbody>
</table>

*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
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/mm³

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/mm³

WHO 2019 policy update on LF-LAM (https://www.who.int/publications/i/item/9789241550604)
• 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*
• 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/mm³ 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

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
The test is performed manually by applying 60 µL of urine to the Determine TB LAM Ag test strip and incubating at room temperature for 25 minutes. The strip is then inspected by eye. The intensity of any visible band on the test strip is graded by comparing it with the intensities of the bands on a manufacturer-supplied reference card. Prior to January 2014, this reference card included five bands (grade 1 representing a very low intensity band to grade 5 representing a high/dark intensity band). After January 2014, the manufacturer revised the reference bands to contain only 4 grades, such that the band intensity for the new grade 1 corresponded to the previous reference card band intensity for grade 2.

GLI practical guide on LF-LAM: Procurement

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

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

<table>
<thead>
<tr>
<th>Equipment needed</th>
<th>Global Drug Facility catalogue description and product code number</th>
<th>Units per pack</th>
<th>Cost in catalogue (2020)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine collection cups</td>
<td>Specimen collection cups, 80 mL each</td>
<td>1,000</td>
<td>US$ 83.30</td>
</tr>
<tr>
<td></td>
<td>GDF product code: 106525</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pipette capable of delivering 60 μL</td>
<td>Pipette capable of delivering 10–100 μL</td>
<td>1,000</td>
<td>US$ 226.94</td>
</tr>
<tr>
<td></td>
<td>GDF product code: 106055</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disposable pipette tips</td>
<td>Pipette tips capable of delivering 10–100 μL (1,000 tips/pack)</td>
<td>10 × 96</td>
<td>US$ 72.75</td>
</tr>
<tr>
<td></td>
<td>GDF product code: 106388</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dual-bulb micropipette³</td>
<td>Dual-bulb Pasteur pipettes with volume of 60 μL for exact transfer of sample</td>
<td>Not available</td>
<td>Not yet available. Prices will be published in the catalogue.</td>
</tr>
<tr>
<td></td>
<td>Non-graduated, non-sterile pipettes can be used</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Timer</td>
<td>Mechanical timer</td>
<td>1,000</td>
<td>US$ 1.11</td>
</tr>
<tr>
<td></td>
<td>GDF product code: 106570</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

GDF: Global Drug Facility.

³ 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.
ACKNOWLEDGEMENTS:

- Kathleen England
- Lice González-Angulo
- Wayne van Gemert
- Petra de Haas
- Patricia Hall
- Heather Alexander
- MSF (Helena Huerga)
- Marcela de Melo Freitas
- Elisa Tagliani
- GLI Core Group
- WHO Global TB programme
- FIND