**Clinical Chemistry** 64:8
1133–1135 (2018)

**Perspective**

# Urine Lipoarabinomannan for Tuberculosis Diagnosis: Evolution and Prospects

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Tuberculosis (TB)[4] affects over 10 million people and kills 1.7 million each year. This devastating epidemic is unacceptable, as, given correct diagnosis and timely initiation of treatment, TB is curable. Unfortunately, case detection is the weakest step in the cascade of care, and about 40% of TB patients are either not diagnosed or not reported to the health system. This is partly attributable to limitations of existing diagnostics, which are either inaccurate or inaccessible, particularly at the primary care level, where most patients begin seeking care with nonspecific symptoms such as cough and fever.

A simple diagnostic test that can be performed at the point of care (POC) in primary care settings has been near the top of the TB community's wish list for many years, and the WHO has published target product profiles (TPPs) with detailed specifications for such a test *(1)*. While no existing TB test meets all POC TB test TPP requirements, the rapid urine lipoarabinomannan (LAM) detection test comes close because of its simplicity, low cost, and ability to be used in decentralized settings.

LAM is a component of the cell wall of *Mycobacterium tuberculosis*, the causal agent of TB, and only one LAM-based commercial assay currently exists, the Determine TB LAM Ag (Alere Inc.). The Determine TB LAM Ag is an inexpensive lateral flow assay that detects LAM from a urine sample in 30 min. The assay is noninvasive and does not require a laboratory or technical equipment. The healthcare worker places 60 $\mu$L of a urine sample onto one end of the strip test and a positive result appears at the other end as a band within 30 min.

Initially, it was hoped that this rapid test might meet the widespread need for a POC TB test. But studies showed low overall sensitivity, with somewhat better accuracy in people living with HIV (PLHIV) who have low CD4+ T-cell counts *(2)*. Despite the low overall accuracy, because TB poses such a high risk of death in severely sick and immunosuppressed patients, the WHO endorsed the rapid LAM test in 2015 *(2)*. According to the WHO policy, rapid LAM testing should only be used to assist in the diagnosis of TB in PLHIV with low CD4 counts (<100 cells/$\mu$L) or HIV-infected patients who are seriously ill. LAM testing should not be used as a screening test for TB *(2)*.

Since its endorsement by WHO, several studies have shown that LAM-positive children and adults with HIV-infection have higher disease severity and are at greater risk of mortality *(3)*, and 1 randomized trial has shown that LAM testing and treatment can reduce mortality in HIV-positive, hospitalized patients with severe illness, advanced immunosuppression, and an inability to self-expectorate sputum *(4)*. One explanation is that LAM in the urine is a marker of higher mycobacterial load and greater disease dissemination and severity, and rapid treatment of such patients is life-saving.

Multiple hypotheses have been proposed to explain how LAM enters the urine. Previously, free renal filtration of LAM through the glomerular membrane was thought to explain positive LAM results. More recently, renal- and blood-disseminated TB in deceased HIV patients have been observed in autopsy studies. In TB-HIV coinfected patients, TB disease is usually very severe, and infection disseminates through the body. Once TB has disseminated to the kidneys, LAM release within the renal system can occur and thus a positive result when tested. However, not all people who are urine LAM positive have renal TB, suggesting that other mechanisms that are not well understood may be at work.

Despite WHO endorsement and patient advocacy for scale-up, uptake of the rapid LAM test has been poor. Patient advocates and various stakeholders have been trying to bring attention to this concern, as they want HIV-infected TB patients to benefit from this potentially life-saving test in hospitalized PLHIV with TB.

Although the future of the current rapid LAM test is uncertain, the field has continued to evolve, with innovative approaches being attempted to increase the sensitivity of urine LAM detection. Several groups and companies are currently working on a high-sensitivity LAM-based test, but the study by Luisa Paris and colleagues is the first concrete proof-of-concept that this is feasible *(5)*.

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Received February 2, 2018; accepted March 9, 2018.
Previously published online at DOI: 10.1373/clinchem.2018.286625

[4] Nonstandard abbreviations: TB, tuberculosis; POC, point of care; TPP, target product profile; LAM, lipoarabinomannan; PLHIV, people living with HIV.

**Table 1. Comparison of commercial Alere® Determine LAM TB assay vs the high-sensitivity LAM detection method of Paris et al. with copper dye and nanocage.**

| | Determine LAM TB test (Alere Inc.) | High-sensitivity LAM (Paris et al., 2017) |
|---|---|---|
| WHO endorsement | Yes (only in HIV-infected patients with low CD4 counts) | No |
| Sample type | Urine | Urine |
| Sample processing and assay procedure | Unprocessed urine; simple, rapid diagnostic test | Incubation, centrifugation, washing, elution, spotting, staining with anti-LAM antibody, secondary antibody staining, chemiluminescence |
| Time to result | 30 min | Not reported |
| Assay cutoff | Approximately 500–1000 pg/mL ("Grade 1" on test) | 14 pg/mL |
| Sensitivity in clinical studies | 44% (95% CI, 31%–60%) (3) | 96% (no CI reported) |
| Specificity in clinical studies | 92% (95% CI, 83%–95%) (3) | 81% (no CI reported) |
| Specialized training of test operator required to run test? | No | Yes |
| Specialized equipment required to run test? | No | Yes |
| Additional reagents required to run test? | No | Yes; hydrogel nanocage with incorporated copper-based reactive dye, buffer containing organic solvents, antibody reagents |
| Useful for diagnosis of TB in HIV-negative people? | No | Potentially, if translated into a clinical test |
| Useful for diagnosis of TB in PLHIV? | Yes | Potentially, if translated into a clinical test |
| Data on correlation between LAM positivity and mycobacterial burden | Yes | Yes |
| Evidence on reduction in patient mortality, based on rapid LAM test and treatment | Yes (5) | No |
| Cost | Low (approximately $3.5 per test) | Unknown |

In December 2017, Paris and coworkers published their work on a LAM-based assay for TB in *Science Translational Medicine (5)*. Hospitalized patients and healthy controls in Peru provided urine samples, and TB diagnosis as well as disease severity were ascertained by conventional methods. Urine samples were processed by centrifugation, with the supernatants retained for analysis. To improve the detectability of LAM, Paris and colleagues made use of a copper-based reactive dye that binds LAM with very high affinity. The current laboratory assay includes a large number of steps: (*a*) incubation of urine with dye-containing nanocages to bind urinary LAM, (*b*) separation of nanocages by centrifugation, (*c*) 3 washing steps, (*d*) elution with a SDS/2-mercaptoethanol buffer and heat treatment, (*e*) centrifugation, (*f*) spotting of the supernatant on a polyvinylidene difluoride membrane, (*g*) staining of the spotted LAM with an anti-LAM mouse monoclonal antibody, (*h*) incubation with a labeled secondary antimouse antibody, and (*i*) detection of chemiluminescence with an instrument. Paris and colleagues presented concepts to simplify this assay.

When assayed in 48 HIV-negative people living with TB vs a group of 53 diseased individuals and healthy volunteers, sensitivity was 96% and specificity was 81%, with an assay cutoff at 14 ρg/mL *(8)*. Interestingly, the study found that urinary LAM was increased in patients with a higher mycobacterial burden, a phenomenon consistent with previous studies using the Determine LAM test *(3)*.

The nanocage and copper dye technique may be a promising step forward in the evolution of LAM detection as it demonstrates the potential of LAM detection in general TB populations, but there is a very long way to go for primetime clinical use. In its current configuration, it is not deployable in primary healthcare settings where a POC test is most needed (Table 1). Substantial product

development will be required to move this LAM detection technology toward a standardized, commercial kit. Ideally, as per the TPP, a high-sensitivity LAM test would not require additional equipment or complex sample processing steps. Additionally, from the ROC plot, it appears that further improvements to the sensitivity will result in a relatively large loss of specificity *(5)*; this trade-off might impact the use case of the test (i.e., rule in vs rule out).

Once a commercial test is developed, adequate evaluation in properly designed field studies will be necessary. Such studies should benchmark performance against published TPPs, and if the evidence is compelling, then it could be considered for policy. Complementary studies considering test cost, cost-effectiveness, and patient impact will all assist in policy development and uptake by countries.

The fact that there is already WHO endorsement and policy developed for the Determine LAM test, and the fact that over a dozen studies now show strong correlation between urine LAM levels and disease severity, is a strong precedent for further research and development, including validation of the technique of Paris and colleagues and other next-generation LAM tests that are under development.

Stakeholders in the TB community, particularly donors and policy makers, may be more inclined to support and fund research that is related to an already-endorsed diagnostic test. Thus, presenting high-sensitivity LAM as a "fast-follower" to the already existing LAM test will be a smart strategy to spur further innovation and uptake.

**Author Contributions:** *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

**Authors' Disclosures or Potential Conflicts of Interest:** *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

**Employment or Leadership:** None declared.
**Consultant or Advisory Role:** M. Pai, the Scientific Advisory Committee of Foundation for Innovative New Diagnostics, Geneva.
**Stock Ownership:** None declared.
**Honoraria:** None declared.
**Research Funding:** None declared.
**Expert Testimony:** None declared.
**Patents:** None declared.

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