Review Article

Antigens of *M. tuberculosis* with reference to diagnosis of the disease with special emphasis on Lipoarabinomannan (LAM)

Pooja Chaudhary\(^a\)\(^c\), Arun P. Sikarwar\(^b\), K. K. Mohanty\(^c\), Shripad A. Patil\(^d\)

\(^a\) Department of Zoology, Dayalbagh Educational Institute (Deemed to be University), Agra, India. E-mail: chaudharypooja9614@gmail.com

\(^b\) Assistant Professor, Department of Zoology, Dayalbagh Educational Institute (Deemed to be University), Agra, India. E-mail: arunsikarwar@dei.ac.in

\(^c\) Scientist-F, Department of Immunology, National JALMA Institute for Leprosy & Other Mycobacterial Diseases, Indian Council of Medical Research (ICMR), Agra, India. E-mail: mohanty.kk@icmr.gov.in

\(^d\) Professor, Department of Neuromicrobiology, National Institute of Mental Health and Neurosciences (NIMHANS), Hosur Road, Bengaluru, India.

E-mail: shripadpatil@yahoo.com

For Correspondence, E-mail: shripadpatil@yahoo.com

Abstract

Tuberculosis (TB) is one of the contagious and notorious diseases globally and there are many tests available for the detection of TB but have severe limitations. There is no reliable test present that can detect TB in an early stage in a quick time and also may discern between stages of TB. Detection of the disease is the weakest step or else it may help in the initiation of early treatment and thus controlling the further spread. Discovering methods to detect TB
are continuously evolving to achieve rapid, cheaper, sensitive, and specific results. Here, we review MTB lipoarabinomannan (LAM) as a diagnostic marker, which is present inside the body fluids of infected subjects. The LAM is not only present in sputum, but also in some other body fluids including urine and blood. Thus, it could be an innovative approach as an alternative to the diagnosis of childhood TB by using urine as a sample. There is urgency for developing new diagnostic tools to detect TB and by using LAM as a diagnostic marker, we can overcome the shortcomings of present tools and techniques. The application of rapid LAM test has the potential to evolve with innovative approaches being attempted to increase the sensitivity of TB detection.

**KEY WORDS:** Tuberculosis, lipoarabinomannan, marker, sensitivity, diagnostic, potential, and childhood.

**Tuberculosis: The Major Killer of Microbial Infection**

Mycobacterium tuberculosis (MTB) is the causative agent of the airborne disease tuberculosis (TB). The MTB is one of the most ubiquitous pathogens over the globe for thousands of years. The MTB was discovered by German physician Robert Koch in 1882. TB is an airborne infectious disease that generally affects the lungs with symptoms of coughing, chest pain, anorexia, and fever. The reason behind its severity is the release of microscopic droplets from a TB patient through sneezing, coughing, or speaking, and subsequently the droplets may spread the disease to a healthy person (1). The size of droplets is up to 5μm, and it may contain up to 1-3 bacilli. Infection of TB is characterized by either latent or active TB. Most of the infected subjects remain asymptomatic i.e., latent TB and infection prevails in a quiescent form without any clinical symptoms of the disease. Epidemiological studies of TB in both developing and developed countries report that 5-10% of latent subjects may develop
active TB during their lifespan (2). Active TB is the condition of an infected subject, when the immune system of a subject is unable to find or defend against MTB. Active TB is one of the disastrous disease and is of great concern due to voluminous human mortality in the world.

About 23% world population is being reported to be infected with MTB which represents almost 1.4 million deaths every year (3). Unfortunately, the case of identification is the most fragile during care, and up to 40% of TB subjects are either not diagnosed or reported on time to medical care. This is to some extent inevitable due to impediments to existing diagnostic methods (4). Multi-drug resistant (MDR), notoriously drug-resistant TB, and progressing pestilence of co-infection of HIV-TB further exacerbate TB management. World Health Organization had declared tuberculosis as a global public health emergency in 1993 and even after decades of persistent global fight, TB is still among one of the top ten causes of global human mortality (5).

TB has been associated with destitution and poverty. The lack of proper health services, malnutrition, social disruption, inadequate living conditions further take along the dissemination of TB. Human immunodeficiency virus (HIV) infection leading to Acquired Immune Deficiency Syndrome (AIDS) is one of the firm and a strongest risk factor for TB.

It is being reported that globally 10 million people were infected with TB in 2019 and there were an estimated 1.2 million TB deaths among HIV-negative and 208,000 deaths among HIV positive subjects (6). In 2014 "post-2015 global TB strategy" was announced by the World Health Assembly, a decision-making body of WHO, to eradicate global TB epidemic with targets to reduce TB mortality by 95% and taking down TB cases by 90% by 2035 (7). Detection of disease in an early phase and providing initial treatment to the patients is the
first segment of this scheme initiative. Therefore, the prevention of disease transmission is major significance and requires early diagnosis, along with appropriate medical treatment.

1. **TB Causing Pathogen: *Mycobacterium tuberculosis***

*Mycobacterium tuberculosis* (MTB) of Mycobactriaceae family is a pathogenic bacterium of TB. The bacteria of this cohort were named so because of the mold-like (myco: fungus; bacterium: bacteria) pellicular growth pattern of these organisms in a liquid medium (8). The genome size of MTB is about 4 million base pairs which contain ~4,000 genes (9). Due to the presence of mycolic acid, the MTB has an unusual waxy coating on its cell surface. Due to this coating, the cells are impervious to gram staining and hence acid-fast dye is used to identify MTB by microscopy (10).

Due to presence of thick peptidoglycan, MTB is categorized as acid-fast and gram-positive. MTB divides in about 18-20 hours and stays in the human alveolar macrophages. TB infection may occur due to either of activation of already existing latent bacilli or by new bacilli. In general, an equilibrium sets up between active and latent bacilli, however, overcoming of the equilibrium causes acute infection (11). As per dynamic reinfection hypothesis, non-replicating bacteria may reach to bronchial tree and initiates infection, followed by the granuloma formation (12). The MTB cell wall is composed of approximately 60% of lipids. Mycolic acid, cord factor, and wax-D comprise a major fraction of MTB cell wall (13). The cell wall of MTB is composed of two segments including the outer part and core of the cell wall (figure 1). The core of the cell wall is made up of peptidoglycan (PG), covalently attached with arabinogalactan (AG) and mycolic acids, forming the mycolyl arabinogalactan peptidoglycan (mAGP) complex. The different cell wall proteins, including phosphatidylinositol mannosides (PIMs), Lipomannan (LM), and Lipoarabinomannan (LAM), are present in the upper part, which is made up of free lipids (14). There are plenty of
antigens present in MTB bacteria and some major antigens are cord factor, Total Mycolic acid-containing Glycolipid (TBGL), sulpholipid-I, and LAM.

![Cell wall envelope of Mycobacterium tuberculosis showing Lipoarabinomannan](image)

**Figure 1.** Cell wall envelope of *Mycobacterium tuberculosis* showing Lipoarabinomannan (Courtesy: Riley L.W., J Clin Invest. 2006, ref 15).

2. **Host-pathogen interaction: Role of host immunity**

In subjects exposed to MTB, the interaction between the host and pathogen is significantly influenced by host immunity. The entry route of tubercle bacilli into the body is via the inhalation of respiratory droplet nuclei (16). Based on the immune response of the host upon exposure to MTB, the exposed human being may get rid of bacteria, may develop an active TB, or expand into a chronic infection without clinical manifestations. The prefatory
reaction is due to the interplay of the specific glycolipids/lipids/carbohydrates and/or peptidoglycans of the MTB cell envelope, with the cells of the innate immune system e.g., macrophages and dendritic cells (17). The way in which macrophages and dendritic cells either activate or suppress distinct antibacterial mechanisms, the cytokines pattern being secreted, and how antigens interact with the major histocompatibility complex (MHC), directs the profile of the acquired immune response. The evoked acquired immune responses mediated through T-cells, perform an especially important part in MTB infection control (18). However, elaborated profile of the host immune response necessary for effective acquired immunity to MTB antigens is yet to be elaborated. The research on acquired immune response revolves around the function performed through antigenic peptides and little addresses the mycobacterial antigens of a lipoglycan nature. The lipoglycan antigens may have been undervalued and may in fact play a vital role in the overall immune response to bacterium and as such be importance for TB diagnosis. While the significance of T-cell immunity has been established considerably, however, the role of humoral immunity has been considered to some extent (19). Numerous pieces of evidence support the role of antibodies and B-cells in the establishment of an efficient immune response against tuberculosis infection (20).

LAM inhibits multiplication of T-cells and bactericidal activities of macrophages (21). LAM molecules can insert themselves into biological membranes and bind with Toll-like receptors (TLRs), affecting signalling events. The ManLAM has been reported to have immunosuppressive nature by restoring IL-10 production and it inhibits the production of IL-12 interfering with TLRs and tumour necrosis factor (TNF). The ManLAM additionally modulates MTB-induced apoptosis of macrophages with the support of binding with host
mannose receptors and that is foremost in deactivating host macrophages to allow bacteria to survive and multiply inside them (22).

3. An urgent need for an improved novel biomarkers for TB diagnosis

The need for a novel TB biomarker at any stage of TB diagnosis, treatment, and prevention is extensively recognized. At present the markers and tests available for TB diagnosis have grave limitations, and hardly anyone is for point-of-care (POC) diagnostic purpose. There is a lack of diagnostic biomarkers as well as predictive markers to test the development of latent to active tuberculosis (23). Available tests are not able to differentiate between subclinical progressing infection and non-progressing latent infection (24). Active TB diagnosis is primarily based on the detection of bacilli in sputum through “sputum smear acid-fast staining” and bacilli culture. Microscopy is largely available; however, missing sensitivity and specificity remain a problem in several samples. The gold standard for diagnosing TB is still mycobacterium culture, but being time-consuming it may require as many as 6-8 weeks for obtaining results (25). The Xpert MTB/RIF test is an automated and cartridge-based system, but the disadvantage side is that it is costly and frequently unavailable in primary-care settings due to the fact of their fundament needs. Diagnosis of latent TB is carried out by tuberculin skin test (TST) or the interferon-γ release assays (IGRAs). The TST makes use of purified tuberculin derivative (PPD), however it may be nonspecific. The TST is primarily based on skin infiltration brought about through intradermal injection of PPD in a crude aggregate of antigens, where lots of these are shared through MTB, *M. bovis*, BCG, and numerous environmental mycobacteria species (26). Neither diagnostic test displays the activity of the infectious focus or the chance of development to active TB. This is a vital shortcoming, as efficient and accurate detection of those with LTBI is at higher risk of
succumbing TB disease in due course of time (27). Although these tests are helpful in patient-management but have insufficient predictive value for progression to active tuberculosis (24).

Indeed, there are so many strategies and techniques for TB diagnosis, but all these are not specific and sensitive for the diagnosis of TB, hence novel solutions for TB diagnosis are needed (table 1). The WHO has described the overall performance and operational characteristics of a test appropriate for primary care or at the POC in its high-priority target product profiles (TPPs) (28). Thus, better biomarkers predictive of TB outcomes are the need of the hour. This is a concern for the TB research and has the potential to affect not only research but also clinical practice globally (29).

Table 1: Currently used TB diagnosis techniques and their limitations.

<table>
<thead>
<tr>
<th>Testing indication</th>
<th>Currently employed technique</th>
<th>Limitation of the current technique</th>
<th>Desirable new technique</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Diagnosis of Latent TB Infection (LTBI)</td>
<td>2. Tuberculin skin test (TST). 3. Interferon-gamma release assay (IGRA)</td>
<td>IGRA and TST are unable to satisfactorily distinguish between latent and active TB. The test is unable to identify those at higher risk of progression to active TB.</td>
<td>A new test that can resolve the spectrum of TB, and identify subjects infected with latent TB who are at higher risk towards progression to active TB and may benefit from preventive therapy.</td>
<td>(30)</td>
</tr>
<tr>
<td>2. Diagnosis of active pulmonary TB</td>
<td>1. Sputum smear microscopy (SSM) 2. Nucleic acid amplification tests (NAAT) 3. Culture</td>
<td>1. Smear microscopy is insensitive and cannot detect drug resistance TB. 2. NAAT test is costly and not easily adaptable at the peripheral level. 3. Culture is extended performance and takes extended time.</td>
<td>A non-sputum-based biomarker test for all kinds of TB needed, as well as sputum-based replacement test for smear-microscopy.</td>
<td>(31)</td>
</tr>
</tbody>
</table>
3. Test to identify individuals with expected TB who need confirmatory testing

| 1. TB symptoms (e.g., two weeks of cough, irregular weight loss). 2. Chest X-ray | 1. Symptoms lack sensitivity and specificity, particularly in HIV-infected subjects and young children. 2. Although sensitive, chest x-rays are not specific for TB. | A simple, inexpensive triage test which is ideal for use by community health workers and might be used as a rule out test by first care-contacts care healthcare providers. |

4. Diagnosis of Extra pulmonary and children TB.

| 1. Smear microscopy 2. Nucleic Acid Amplification Test (NAAT). 3. Culture. | 1. Paediatric patients with EPTB often are unable to produce sufficient sputum. Invasive samples are usually necessary. Smear microscopy does not have appropriate sensitivity and specificity. 2. NAAT tests are cost effective and not easily adaptable at the peripheral level. 3. Culture is time consuming. | For all TB types (pulmonary and extra pulmonary TB), a non-sputum-based biomarker test is required. |

4. Lipoarabinomannan as a potential Diagnostic marker for TB

LAM is one of the major lipoglycan of the mycobacterial cell envelope and has been proven to be present inside the body fluids of MTB-infected individuals. Post mycobacterial infections, LAM molecule is present in many body fluids making it a potential biomarker to identify infection. Also, the immune response against LAM can also serve as a diagnostic tool.

For all TB types (pulmonary and extra pulmonary TB), a non-sputum-based biomarker test is required. (31, 32)

Mycobacterium has a peculiar cell wall with an array of lipid-based molecules that provide a thick waxy surface. One of major components of cell envelope is LAM, which is a major antigenic glycolipid is an essential immunodiagnostic goal for detecting TB infection (33). LAM is a significant structural element of the mycobacterium cell wall and is a prominent
mediator of functions that result in successful infection and pathogenicity (17). Antigenic molecules in the LAM are made up of repeating five linked D-arabinofuranose residues. Epitopes on this molecule are arranged on the surface of the mycobacterium cell wall, however in *M. leprae*, these have an inside orientation. LAM is soluble in water, resistant to proteases and boiling and is believed to be degraded slower than protein molecules because of its polysaccharide nature (34). LAM is a glycoconjugate and is one of virulence element associated with MTB. Having been as one of wall component, it allows the MTB to survive in the host cell by affecting equilibrium of host resistance and immune responses. It is being reported as 15% of the total weight of bacteria. LAM of mycobacterium is 17.3 kDa as reported by several MALDI-MS studies (35). LAM molecule is made up of three components: a phosphatidylinositol membrane anchor, a (1→6)-linked mannan backbone of mannopyranose (Manp), and an arabinan chain containing a couple of arabinofuranoside (Araf) residues with and hexa-Araf termini. At the time of infection, the membrane anchor attaches the molecule to the cell wall, and homo polysaccharides functions as carbohydrate skeleton (36) (figure 2). There are three major classes of the LAM based on the presence and structure of capping. In mannosylated LAMs (Man LAM), the mannosyl groups are present on the D-arabinan group. After the mannosyl capping, the Man LAM acts as an anti-inflammatory molecule and inhibits the production of TNF-α and IL-12. Such properties of Man LAM facilitate the bacteria to survive in the host cell for a long time (37). The Man LAMs are observed in pathogenic mycobacterial species including MTB, *M. leprae*, and *M. bovis*. Phosphoinositol-capped LAMs (PILAM) are LAMs capped with phosphoinositol groups and are present in non-pathogenic *M. Smegmatis* (38).

Arabinofuranosyl-Terminated LAM (Ara LAM), Ara LAM 1, and 3-mannosyl side chains are found in many mycobacterial species. Several lipid additives of bacterial cell wall e.g.,
Lipomannan (LM) and phosphatidylinositol mannosides (PIMs), are being found in the synthesis of LAM. The LAM is synthesized with the aid of using the addition of mannopyranosyl to a phosphoinositol. The PIMs are taken into consideration as pioneers of LAMs in the biosynthesis pathway (21). The PIMs and LM are synthesized by the addition of mannopyranosyl to a phosphoinositol. Glycosylation of PIMs and LM with arabinan forms LAM (39). Mannosyl transferases are involved in the synthesis of PIMs.

Figure 2. Structure for lipoarabinomannan (LAM) with typical carbohydrate composition of MTB ManLAM (Courtesy: Turnbull W.B. et al., Org & Biomol Chem, 2012, ref 40).
The concentration of LAM inside various body fluids can be influenced by other factors including bacterial load, co-infection with HIV, and/or site of infection. The HIV-TB co-infected patients with immune suppression and disseminated TB have been reported with higher LAM concentrations in urine (41). The detection of MTB pathogen markers has a potential to test with higher specificity than host markers.

Simple antigen detection has the capability to use as a diagnostic marker for TB because the diagnosis of MTB by DNA-based method is an achievement, but this is cost-effective and complex (42). There is a lot of literature available on the immunogenic features of LAM and on its antigenic attributes, and hence LAM is abundant and antigenic in nature making it very crucial for the diagnosis of TB (table 2). Our laboratory has been working to use it as TB diagnostic marker.

Table 2: LAM as the diagnostic maker for tuberculosis.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Title of Paper</th>
<th>Study Type</th>
<th>Sample type</th>
<th>Total No. of samples</th>
<th>Technique Applied</th>
<th>Location of Sample Collection</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Year</th>
<th>Outcome</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Diagnostic accuracy of a urine LAM-ELISA for screening ambulatory HIV infected persons for TB</td>
<td>Cross-sectional study</td>
<td>Sputum, Blood, Urine</td>
<td>422</td>
<td>AFB, mycobacterial culture using BACTEC MGIT 960 system and ELISA</td>
<td>Tembisa Main Clinic in Ekurhuleni, South Africa.</td>
<td>LAM-ELISA Sensitivity 32%</td>
<td>Specificity 98%</td>
<td>2011</td>
<td>Sensitivity of the LAM in urine was lower than that reported in previous studies, likely because the participants of that study were ambulatory and some sick persons with lower bacillary</td>
<td>43</td>
</tr>
<tr>
<td>2.</td>
<td>A bispecific antibody-based assay shows potential for detecting TB in resource constrained laboratory settings</td>
<td>N/A</td>
<td>Serum</td>
<td>21 sample (14 were TB positive and 7 were negative)</td>
<td>Immuno-swab assay, SDS-PAGE, western blot, FACS and sandwich ELISA</td>
<td>TB samples from TB Trials Consortium (TBTC) and healthy controls from Fort Collins, CO, U.S.A.</td>
<td>Assay showed Sensitivity -64% &amp; Specificity 100%</td>
<td>2012</td>
<td>The assay might be used as a rapid diagnostic tool in resource constrained laboratory settings, as this assay has all the characteristics of an ideal diagnostic TB test as affordable, more sensitive, specific, user-friendly, rapid, and equipment-free and can be delivered to those in need.</td>
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<td>3.</td>
<td>The value of serum LAM in the diagnosis of pulmonary TB</td>
<td>Case-control study</td>
<td>Serum</td>
<td>40 PTB and 20 healthy</td>
<td>Ziehl-Neelsen (AFB), LAM - ELISA</td>
<td>Chest and Medical Biochemistry Department, Faculty of Medicine, Menoufia University Hospitals, Egypt.</td>
<td>Sensitivity: ELISA-90% ZN smear-85% Specificity: ELISA 100% ZN smear-</td>
<td>2014</td>
<td>The LAM test is simple, reliable, and rapid diagnostic test for the PTB. The LAM serum assay test is unlikely to be used alone for definitive TB diagnostic</td>
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</tbody>
</table>
### Table 4: Detection of LAM in urine

<table>
<thead>
<tr>
<th>No.</th>
<th>Description</th>
<th>Meta analysis</th>
<th>Urine</th>
<th>N/A</th>
<th>100%</th>
<th>2016</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.</td>
<td>Detection of LAM in urine is an independent predictor of mortality risk in patients receiving treatment for HIV-associated TB in sub-Saharan Africa: a systematic review and meta-analysis.</td>
<td></td>
<td></td>
<td>1172</td>
<td>N/A</td>
<td></td>
<td>This study has proven that HIV-TB co-infected patients and detectable urinary LAM patients have greater mortality rate as compared to TB patients without detectable urinary LAM.</td>
</tr>
</tbody>
</table>

### Table 5: The diagnostic value of urine LAM antigen in childhood TB

<table>
<thead>
<tr>
<th>No.</th>
<th>Description</th>
<th>Cross-sectional study</th>
<th>Urine</th>
<th>Paediatric patients 61 suspected either PTB &amp; ETB, aged 0-14 years.</th>
<th>Microbiological examination (AFB staining, Sputum culture) and ELISA</th>
<th>Child Health Department of Saiful Anwar Hospital in Malang, East, Java, Indonesia</th>
<th>Sensitivity: ELISA-83%, Microbiological test- 33% Specificity: ELISA-85%, Microbiological test- 60%</th>
<th>2017</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.</td>
<td>The diagnostic value of urine LAM antigen in childhood TB</td>
<td>Cross-sectional study</td>
<td>Urine</td>
<td>Paediatric patients 61 suspected either PTB &amp; ETB, aged 0-14 years.</td>
<td>Microbiological examination (AFB staining, Sputum culture) and ELISA</td>
<td>Child Health Department of Saiful Anwar Hospital in Malang, East, Java, Indonesia</td>
<td>Sensitivity: ELISA-83%, Microbiological test- 33% Specificity: ELISA-85%, Microbiological test- 60%</td>
<td>2017</td>
<td>Urinary LAM provides a few benefits due to the fact samples are easy to collect compared to sputum collection. Rapid urinary LAM test result is expected to improve childhood TB control as a POC test and...</td>
</tr>
<tr>
<td>6.</td>
<td>Diagnosing TB in hospitalized HIV-infected individuals who cannot produce sputum is urine lipoarabinomannan testing the answer?</td>
<td>Multi-centre study</td>
<td>Sputum, Urine</td>
<td>Total patients enrolled 2528 cohort 1- Sputum producing 2341 samples. cohort 2- 187 sputum scarce</td>
<td>Sputum culture, chest X-ray, and Alere determine TB LAM Ag lateral flow</td>
<td>South Africa, Tanzania, Zambia, and Zimbabwe</td>
<td>N/A</td>
<td>2017</td>
<td>This study states that urine LAM testing facilitates rapid diagnosis and positive predictive value in hospitalized HIV patients with scarce sputum. This POC test is a useful diagnostic tool of TB in those patients who cannot produce enough sputum.</td>
</tr>
</tbody>
</table>

| 7. | Detection of LAM in urine and serum of HIV-positive and HIV-negative TB suspects using an improved capture-ELISA and | Cohort study | Urine, Serum | Cohort 1- 25 TB & HIV +ve, cohort 2- 25 TB +Ve HIV – Ve, cohort 3. | Capture ELISA, gas chromatography/Mass spectrometry (GC-MS) | Vietnam, South Africa, and Peru | Sensitivity: GC/MS - 99% ELISA – 98% Specificity: GC/MS – 84% ELISA – 92% | 2018 | The GC/MS and ELISA have a significantly better sensitivity and specificity and confirmed that LAM is present in HIV-ve and TB+ve patients in lower amounts, than HIV +ve/TB +ve. |
| Biomarker for TB: case for lipoarabinomannan | GC/MS | 25 TB - Ve HIV +Ve, Cohort 4. 25 TB & HIV - Ve | N/A | N/A | N/A | N/A | Sensitivity: 1. Clearview test TB-HIV +ve - 51% TB-HIV - ve - 14% 2. Alere test TB-HIV +ve - 45% TB-HIV - ve - ND Specificity: 1. Clearview test HIV +ve 94% HIV +ve - 97% 2. Alere test HIV +ve - 92% HIV -ve - ND | 2019 | A method of detection and quantification of LAM in serum need to be further explored. If increased sensitivity of an Ag test for LAM is achieved, LAM should be investigated as a predictive biomarker of the outcomes following MTB infection as well as a biomarker in TB treatments. | 49 |
9. **LAM in sputum to detect bacterial load and treatment response in patients with pulmonary TB:**
   Analytic validation and evaluation in two Cohorts

<table>
<thead>
<tr>
<th>Case-contr</th>
<th>sputum</th>
<th>308 patients, 244 diagnosed as PTB and 64 diagnosed as non-TB</th>
<th>Smear microscopy, MGIT 960 and LJ culture, LAM-ELISA, and Xpert MTB/RIF</th>
<th>Manila, Philippines</th>
<th>Sensitivity: LAM-ELISA-70% Xpert MTB/RIF-79.3% Specificity: LAM-ELISA-100% Xpert MTB/RIF-ND</th>
<th>2019</th>
</tr>
</thead>
</table>

This study reports that LAM-ELISA can ascertain the LAM concentration in sputum, and LAM in sputum measured by the ELISA may be utilised as a biomarker for bacterial load both before and after TB treatment.

10. **Combining urine lipoarabinomannan with antibody detection as a simple non-sputum-based screening method for HIV-associated TB**

<table>
<thead>
<tr>
<th>Case-contr</th>
<th>Urine, serum</th>
<th>104 (TB cases 74 and randomly selected non-TB 30)</th>
<th>U-LAM, Sputum microscopy, Culture, Gene Xpert</th>
<th>Gugulethu township, Cape Town, South Africa</th>
<th>Sensitivity: U-LAM with Ab detection-92%, Sputum microscopy with IgG detection-88%, Xpert-96% Specificity: U-LAM with Ab detection - 80%</th>
<th>2019</th>
</tr>
</thead>
</table>

Combining urine LAM with serum antibody detection could offer easy low-cost technique that meets the necessities for a non-sputum-based test for the screening of HIV-associated tuberculosis.

11. **Lateral flow Prosp Urine 280 Alere Siriraj Hospital Sensitivity 2019**

The LF-LAM
<p>| 12 | Novel urine lipoarabinomannan Assay for diagnosis of active TB in adults with human immunodeficiency virus infection: a prospective cohort study | Urine specimens from 968 hospitalized patients with HIV | FujiLAM and AlereLAM Assay | FIND specimen bank and the university of Cape Town, South African hospitals | Sensitivity: FujiLAM - 70.4% AlereLAM - 42.3% Specificity: FujiLAM - 90.8% AlereLAM - 95% | 2019 | FujiLAM gave advanced diagnostic sensitivity, specificity and could transform rapid POC TB diagnosis for hospital in patients with HIV compared to AlereLAM. |
| 13 | Point-of-care urine lipoarabinomannan antigen detection for diagnosis of TB in children | Urine, sputum, or induced sputum from 381 children recruited in Cohort 1-280 children with ZN staining, culture, Xpert MTB/RIF testing, and LAM assay | OPD of the Department of Paediatrics, All India Institute of Medical Sciences (AIIMS), New Delhi, India | LAM Assay Sensitivity: ITTB patients - 73.2% LNTB patients - 78.6% | 2019 | The LAM assay increased the accuracy of TB detection significantly while in comparison with other reference tests. Urinary |</p>
<table>
<thead>
<tr>
<th></th>
<th>Detection of mycobacterial lipoarabinomannan in serum for diagnosis of active TB</th>
<th>Retropective case-control study</th>
<th>Serum, sputum</th>
<th>145 subjects with clinical symptoms, 90 confirmed PTB and 55 non-TB</th>
<th>Single Molecule Array (Simoa), liquid and solid cultures, AFB and Xpert</th>
<th>Vietnam, South Africa, and Peru</th>
<th>LAM testing showed high specificity and sensitivity in pediatric TB. LAM assay may also show to be useful as new diagnostic tool for pediatric TB.</th>
</tr>
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<tbody>
<tr>
<td>14</td>
<td>Detection of mycobacterial lipoarabinomannan in serum for diagnosis of active TB</td>
<td>Retrospective case-control study</td>
<td>Serum, sputum</td>
<td>145 subjects with clinical symptoms, 90 confirmed PTB and 55 non-TB</td>
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</tr>
<tr>
<td>15</td>
<td>Diagnostic accuracy of 3 urine lipoarabinomannan</td>
<td>Multi-centre diagnosis</td>
<td>Urine and sputum</td>
<td>372 patients included (111</td>
<td>FujiLAM, AlereLAM, EclLAM, SSM,</td>
<td>Healthcare centre in Peru, South Africa, Khayelitsha</td>
<td>Compared with AlereLAM, FujiLAM was detected five times more sensitive.</td>
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<td>mannun TB assays in HIV-negative outpatients</td>
<td>Xpert and MGIT liquid culture and solid culture on LJ media</td>
<td>township, DOTS treatment centre in Suburbs of Lima, University of Cape Towns</td>
<td>Test</td>
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<td>definitive TB, 10 TB and 251 not TB)</td>
<td>Xpert and MGIT liquid culture and solid culture on LJ media</td>
<td>Xpert and MGIT liquid culture and solid culture on LJ media</td>
<td>FujiLAM-53.2%</td>
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<td>Xpert and MGIT liquid culture and solid culture on LJ media</td>
<td>Xpert and MGIT liquid culture and solid culture on LJ media</td>
<td>AlereLAM M-10.8%</td>
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<td>Xpert and MGIT liquid culture and solid culture on LJ media</td>
<td>Xpert and MGIT liquid culture and solid culture on LJ media</td>
<td>EclLAM-66.7%</td>
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<td>Xpert and MGIT liquid culture and solid culture on LJ media</td>
<td>Xpert and MGIT liquid culture and solid culture on LJ media</td>
<td>Sputum Test-SSM-61.3%</td>
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<td>Xpert and MGIT liquid culture and solid culture on LJ media</td>
<td>Xpert and MGIT liquid culture and solid culture on LJ media</td>
<td>Xpert-76.6%</td>
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<td>Xpert and MGIT liquid culture and solid culture on LJ media</td>
<td>Xpert and MGIT liquid culture and solid culture on LJ media</td>
<td>Combine Sputum test+ FujiLAM SSM + FujiLAM-70.3%</td>
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<td>Xpert and MGIT liquid culture and solid culture on LJ media</td>
<td>Xpert and MGIT liquid culture and solid culture on LJ media</td>
<td>Xpert+ FujiLAM-82%</td>
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<td>Xpert and MGIT liquid culture and solid culture on LJ media</td>
<td>Xpert and MGIT liquid culture and solid culture on LJ media</td>
<td>Specificity: Urine LAM Test- FujiLAM-98.9%</td>
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<td>Xpert and MGIT liquid culture and solid culture on LJ media</td>
<td>Xpert and MGIT liquid culture and solid culture on LJ media</td>
<td>AlereLAM M-92.3%</td>
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<td>Xpert and MGIT liquid culture and solid culture on LJ media</td>
<td>Xpert and MGIT liquid culture and solid culture on LJ media</td>
<td>EclLAM-98.1%</td>
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<td>Xpert and MGIT liquid culture and solid culture on LJ media</td>
<td>Xpert and MGIT liquid culture and solid culture on LJ media</td>
<td>Sputum Test-</td>
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<td>Xpert and MGIT liquid culture and solid culture on LJ media</td>
<td>Xpert and MGIT liquid culture and solid culture on LJ media</td>
<td>times more in the patients with TB in HIV-ve subjects and had a high positive prognostic value. This has the potential to boost rapid diagnosis of TB at the POC. EclLAM in contestable that further sensitivity gains are possible, that highlights LAM as a potential biomarker.</td>
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Unedited version published online on 24/7/2023
16. **Point-Of-Care urine LAM tests for TB diagnosis: a status update**

| Revi ew articl e | Urine LAM | N/A | AlereLAM , FujiLAM, Sputum culture, microscopy, NAAT, and Xpert | N/A | N/A | 2020 |

17. **Cost-effectiveness of a novel lipoarabinomannan test for TB in patients with HIV**

| Clini cal cohort study | Sputum, Urine | N/A | 1) sputum Xpert MTB/RIF 2) sputum Xpert+ urine AlereLAM 3) sputum Xpert+ urine FujiLAM and CD4 | South Africa, Malawi | N/A | 2020 |

| SSM-100% Xpert-100%
Combine Sputum test+
FujiLAM SSM + FujiLAM-98.9%
Xpert+ FujiLAM-98.9% |

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Urine LAM is a promising TB diagnostic biomarker. The final objective of a urine LAM test is to achieve high sensitivity and specificity for TB patients.

This study found that combining urine FujiLAM to sputum Xpert for TB testing among unselected hospitalized PWH would increase life expectancy and be economical.
Additional feasibility studies should examined FujiLAM in clinical practice settings.

Abbreviations: TB: tuberculosis; ELISA: enzyme-linked immunosorbent assay; LAM: lipoarabinomannan; +VE: positive; -VE: negative; IgG: immunoglobulin-G; U.S.: United States; AFB: acid-fast bacilli; HIV: human immunodeficiency virus; PTB: pulmonary TB; EPTB: extra-pulmonary TB; MGIT: mycobacterial growth indicator tube; SDS: Sodium dodecyl sulphate; PAGE: polyacrylamide gel electrophoresis; FACS: fluorescence-activated cell sorting; GC: Gas chromatography; MS: Mass spectrometry; POC: point of care; LJ: Lowenstein Jensen medium; MTB: mycobacterial TB; RIF: rifampin; MTB: Mycobacterium TB; ND: not determined; U: urine; LF: lateral flow; Ag: antigen; Ab: antibody; SSM: sputum smear microscopy; ITTB: Intra thoracic TB; LNTB: lymph node TB; NAAT: nucleic acid amplification test; PWH: patient with HIV; DOTS: directly observed treatment, short-course; OPD: outpatient department.

5. Gaps and unmet needs for new diagnostic biomarkers

Up to 40% of TB patients are either not diagnosed or reported to the health system, making TB diagnosis one of the weakest steps in the cascade of care. This is partially due to the restraint of available diagnostic tools, which are either ineffective or inaccessible, particularly at the primary care level, where the majority of patients seek care for non-specific signs and symptoms like cough and fever (4). The world needs newer effective strategies and techniques for managing tuberculosis. A simple diagnostic test with ease of application at the POC in primary care settings has been a dream to TB medical care for plenty of years. The
WHO has published TPPs with elaborated specifications for such a test (59). Nowadays recently posted examinations of various stakeholders has assisted to set up the maximum essential unmet needs and this has helped to become aware of tools of most importance. In interviews and reviews of publications, TB researchers have identified the need for developing numerous TB diagnostic tests in addition to the currently available tools. In the list of triage and screening tests, the assessment for patients is hard to diagnose for the children, TB-HIV co-infected patients, and patients with EPTB with a non-sputum-based biomarker test for diagnosis of active TB (60, 61). A highly sensitive TB test based on biological samples apart from sputum (inclusive of urine, blood, saliva, or exhaled air) relevant for implementation at lower levels of care would be a feasible help and shorten the delay before diagnosis to permit early treatment (62). A non-sputum-based biomarker may be useful in the diagnosis of a latent TB infection that predicts progression to active TB and a test for treatment monitoring of drug-susceptibility tests (DST) executed in decentralized or centralized settings (63). This priority exercise eventually identifies the consecutive test as key priorities:

1. Smear replacement test- A rapid sputum-based test as a substitute for smear microscopy with or without DST. The smear microscopy test is extensively used for TB diagnosis; however its sensitivity limitations are well known.

2. Non-sputum-based biomarker test- rapid non-sputum-based test capable of detecting all kinds of tuberculosis through the identity of traits biomarkers or biosignatures.

3. Triage test- A triage test, which must be a easy, inexpensive to be used with the aid of using by first-contact health care contributors as a rule-out test. This is the test for identification and confirmation for TB.

4. Rapid DST at microscopy centre level.
In these priorities, the second and third tests are especially for progressed diagnosis in young people, which constitute an around 10% of the worldwide TB burden. Eradication of TB cannot be completed without identifying subjects with latent infection who are most at risk of developing active TB disease. The WHO (2014) has published a consensus file with TPPs for priority diagnostics, with illustrations (59). Till now there is no standard TB diagnostic test existing that meets all POC TB test TPP requirements, only the quick urine LAM test comes close to that because of its ease of sample collection, less cost-effective and capability to be used in decentralized settings (4).

6. LAM as novel diagnostic marker for TB

To increase early case detection and address various current gaps in TB control, new diagnostic devices and strategies are being expected in that field. Early diagnosis of tuberculosis is one another important aspect of biomarker research because initially most of the MTB-infected subjects remain healthy but have latent TB infection (LTBI). Current diagnostic tools for TB rely on sputum samples and suffer a lot of disadvantages and limitations. Several diagnostics strategies and tools are under development. WHO (2014) has referred to the development of a “rapid biomarker-based non-sputum test capable of detecting all forms of TB by identifying characteristic biomarkers” (59).

There are two LAM based commercial assays available i.e., Abbott determining TB LAM Ag (AlereLAM) and Fujifilm SILVAMP TB LAM Assay (FujiLAM). These LAM based tests meet the WHO TPP traits for a biomarker-based, non-sputum TB test but studies show low sensitivity and specificity (57).
Hence, the prime aim of LAM test is to attain high diagnostic precision of sensitivity and specificity. There are so many difficulties and obstacles faced in the diagnostic accuracy of the LAM test. One predominant problem in estimating non-sputum-based tests is an inferior reference standard, mainly for patients with EPTB and adolescent TB. For intensifying these limitations, a microbiological reference standard (MRS) and composite reference standard (CRS) must be taken into consideration with the LAM test (64). MRS needs to combine consequences from extra pulmonary samples and pulmonary samples mycobacterial culture, ZN staining, and Xpert test to confirm definite TB. The CRS is used to make a final diagnosis primarily based on the result of two or more tests in TB CRS combines chest X-ray, clinical suspicion, and treatment initiation to define the definite TB (65). TB diagnosis in children is troublesome because clinical presentation is not specific, chest X-ray explication has low accuracy, and collection of sputum samples is incredibly challenging (66). Urine LAM Ag detection test is a rapid and non–invasive alternative for the diagnosis of childhood TB which means urinary LAM has a great diagnostic value for childhood TB (46). The key factor about LAM is that it can diagnose TB in patients and children who are not capable to produce sputum, therefore the patients not able to produce sputum must now no longer be excluded from any sort of study and this group is likely advantaged by LAM testing using non-sputum urine samples.

It is necessary to evaluate the innovative diagnosis yield of sputum-based diagnostics combined with urinary LAM diagnostics (57). If improved sensitivity of a LAM antigen test is achieved, LAM must be explored as a predictive biomarker of the consequences following MTB, further a marker to evaluate the efficacy of anti-TB therapies (49). As we point out in the last paragraph the LAM antigen detection test comes close to the utmost of the POC TB test TPP needs. So many companies and groups are working on the high sensitivity of LAM
detection and LAM Ag as a diagnostic biomarker might provide a newer perception in the diagnosis of TB. The urine LAM test has spawned amazing enthusiasm (67).

LAM is present in the urine of TB patients and that is proved by the study where mice are injected with the sonicated crude H37RV cell wall, so in keeping with this study’s detection of LAM in urine, we can study it as a diagnostic approach for the identification of active TB (68). There is no technique present to measure the bacterial burden of PTB throughout; however, some research suggested that LAM-ELISA can identify the LAM concentration in sputum samples, so measured LAM concentration in sputum may be a good biomarker of a bacilli burden before treatment and during the treatment (50). Sputum LAM-ELISA assay could provide a real-time monitoring tool for the effectiveness of TB treatment response and useful resources for individualized methods to TB affected person therapy regulation. There are lot of research and studies that introduce LAM antigen as a diagnostic tool for the diagnosis of TB and optimistically it is going to give a newer insight into that field (69). The main purpose for the assessment of LAM as a diagnostic tool is that one may come across to detect LAM in urine, which is non-invasive and easy in handling.

Analysis of the possible impact of bringing LAM into the analytic and care falls need to see LAM testing with regards to its placement in a definite medical care foundation and its pursuit of different reachable diagnostics (57). The development of novel biomarkers, primarily based on non-sputum tests may be essential to eradicate TB from the world. Hence, a novel biomarker for TB test requires an appropriate evaluation and validation earlier than global implementation (65).
LAM can be a new brand approach or technique for the diagnosis of TB within the early stage. The future of the current rapid LAM test has endured evolving, with innovative tactics being attempted to improve the sensitivity of LAM detection.

7. Conclusion and future prospects

Tremendous human TB infection needs a new diagnostic tool that is capable of detecting TB infection in the latent phase and in a brief period (70). There are such a lot of reference standard techniques present for the TB diagnosis like AFB microscopy, sputum culture, and gene Xpert NAAT but quite a few of those strategies have a lot of shortcomings like time-consuming, low sensitivity, and price-effective respectively. New biomarkers for TB diagnosis are important to control the unfolding of the disease. So, there is an immediate need for a new diagnostic tool for TB with the aid of using a novel non-invasive method. An appreciable amount of interest in biomarkers and biosignatures for TB detection is motivating. Another concern is the absence of a non-sputum-based tests for children and biomarker test for development of TB. Increasing investments are important to assist biomarker discovery, evaluation, and validation into clinical tools (71). TB biomarkers research is an area of excessive interest; however, its effect has been so restricted. New independent research is needed for the higher established order of recent new biomarkers for TB. Although funding and interest in biomarker research have increased from basic biomarker studies to fundamental biomarker discovery to clinical applications. Improved detection of lipoglycan biomarker LAM should result in a step forward leading to a breakthrough urine-based LAM antigen detection test.

The detection of TB in paediatric patients is challenging due to insufficient sputum production, however with the aid of urinary LAM Ag test, TB can be diagnosed among
children with presumed TB. According to this study, LAM Ag detection may lead to an early
detection of TB in children (54). LAM is optimistic biomarker for the diagnosis of TB, with
appreciable capacity for adults to young paediatric patients because LAM is present in most
of the body fluids e.g., sputum, urine, blood etc.

Hence, we need to explore LAM as a predictive biomarker for the MTB infection. We would
like to signify that according to review of literature, a diagnostic marker could be helpful in
detecting the disease in the community. Hence, if disease is detected in an early phase, it
would also help in initial treatment and controlling the spread of the disease. Exploit and
scale-up of present LAM tests and unfolding of next-generation assays need to be prioritized.
There is foremost demand for TB biomarkers at all the levels. New biomarkers and bio-
signatures can implement in effective diagnosis, prevention, and prognosis of eradication of
TB.

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Conflicts of interest:

The authors have none to declare.

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