Current Biomedicine
p-ISSN: 2962-8490; e-ISSN: 2985-4784
https://journal.ipb.ac.id/index.php/currbiomed/

Curr Biomed, 2025, 3(1): 7–10
DOI: 10.29244/currbiomed.3.1.7

Research

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Sensitivity and specificity of the lipoarabinomannan test compared to GeneXpert in urine samples for tuberculosis diagnosis

Danni Irawan[1]*, Zuri Rismiarti[2], Acep Tantan[2], Fusvita Merdekawati[2]

[1] Study Program of Medical Laboratory Technology, Politeknik Kesehatan Kemenkes Bandung, Indonesia
[2] Division of Molecular Biology, Politeknik Kesehatan Kemenkes Bandung, Indonesia

Received 7 May 2024 | **Revised** 20 June 2024 | **Accepted** 7 August 2024 | **Published online** 1 January 2025

Abstract

Background *Mycobacterium tuberculosis* (MTB) is the causative agent of tuberculosis (TB), primarily affecting lung tissue but also capable of infecting pleura, lymph nodes, bones, and other extrapulmonary sites. Lipoarabinomannan (LAM) is a critical lipopolysaccharide in the outer wall of mycobacterial cells and can be detected in the urine of TB patients as an antigen.

Objective This study aimed to assess the sensitivity and specificity of the LAM test compared to GeneXpert in urine samples from suspected TB patients.

Methods A quasi-experimental design was employed, where urine samples were collected from patients diagnosed with TB at Sidawangi Lung Hospital, West Java Province. The LAM test was performed on 40 samples by applying 60 µL of urine onto LAM test strips, while MTB presence in urine was examined using GeneXpert.

Results LAM test results showed 32.5% positivity, while 67.5% were negative. GeneXpert results indicated 20% positivity and 80% negativity. The LAM test demonstrated a sensitivity of 100% and specificity of 79.4% compared to GeneXpert, with an area under the curve (AUC) value of 0.897.

Conclusion The LAM test showed high sensitivity and moderate specificity compared to GeneXpert in urine samples of suspected TB patients.

Keywords: GeneXpert | lipoarabinomannan (LAM) test | sensitivity | specificity | tuberculosis (TB)

Introduction

Mycobacterium tuberculosis (MTB) is a bacteria responsible for tuberculosis (TB), primarily infecting pulmonary tissue but also capable of infecting extrapulmonary sites such as the pleura, lymph nodes, and bones. This infectious disease is transmitted through airborne droplets and tends to be chronic (Burhan *et al.*, 2020). According to the World Health Organization (WHO), it was estimated that there were 10.6 million global TB cases in 2021. Of these, 6.4 million (60.3%) were reported and treated, whereas 4.2 million (39.7%) remained undiagnosed or unreported (WHO, 2022).

Accurate diagnosis is crucial for the effective treatment and management of TB. Diagnostic methods must ensure sensitivity, specificity, speed, and the ability to differentiate between the varying pathophysiological spectra of TB. Moreover, clinical specimens should be easily obtainable, and the diagnostic tests used should

*Corresponding author Email: danni.irawan17@gmail.com

ideally be cost-effective and straightforward to interpret (Flores *et al.*, 2021). The gold standard for TB diagnosis remains culture examination using the Lowenstein Jensen medium, which can detect live MTB. In contrast, the GeneXpert rapid molecular test can detect MTB but does not distinguish between live and dead bacteria (Lukitosari *et al.*, 2020).

The GeneXpert system is an automated, user-friendly, and rapid diagnostic tool that employs nested real-time polymerase chain reaction (PCR) and molecular techniques to detect MTB and rifampicin (RIF) resistance (Rivani *et al.*, 2019). MTB DNA can be qualitatively detected in sputum and non-sputum samples using GeneXpert; however, the test is unsuitable for monitoring patients undergoing treatment (Rukmana *et al.*, 2015).

An alternative approach to TB diagnosis is through the detection of TB antigens, such as lipoarabinomannan (LAM). LAM is a major component of the MTB cell wall and a byproduct of bacterial degradation by infected macrophages (Puspita *et al.*, 2021). Once MTB enters pulmonary tissue, it causes infection and initiates an immune response. As the bacteria proliferate, they can enter the bloodstream and disseminate to other organs. When replicating MTB degrades, LAM, a vital component of the MTB cell wall, circulates in the blood, passes through the glomerular basement membrane, and is excreted in urine (Bulterys *et al.*, 2019). Urine samples are more accessible than sputum samples, and handling them poses a lower biohazard risk, as sputum samples can generate infectious aerosols. Moreover, urine samples have fewer contaminants, making them easier for sample handling in the laboratory. Urine-based LAM testing can aid in diagnosing active TB, particularly in TB cases co-infected with HIV (Boenjamin *et al.*, 2017).

Urine-based LAM testing serves as a valuable complement to sputum screening using GeneXpert. It primarily benefits TB patients without respiratory symptoms or who can not produce sputum (Lawn *et al.*, 2017). The sensitivity of urine-based LAM testing is superior to sputum testing in TB-HIV patients with advanced immunodeficiency. Furthermore, urine samples are easily obtained from pediatric and adult patients, stored and processed, and present a lower risk of transmission to healthcare workers (Peter *et al.*, 2010).

Sensitivity refers to a diagnostic test's ability to accurately detect those with the disease, known as the true-positive rate. In contrast, specificity measures the test's ability to correctly identify individuals without the disease, or the true-negative rate. A highly sensitive test yields more true positives and fewer false negatives (Supriyanta & Setiawan, 2021). In 2022, Sidawangi Lung Hospital in West Java Province processed 2,487 sputum samples using GeneXpert. This study evaluated the sensitivity and specificity of urine-based LAM testing compared to GeneXpert among suspected TB patients.

Methods

Study period, location, and design

This study was conducted from September to October 2023 at Sidawangi Lung Hospital, West Java Province. This study has been approved by the Health Research Ethics Committee of Politeknik Kesehatan Kemenkes (Poltekkes Kemenkes) Bandung (No. 39/KEPK/EC/XII/2023). A quasi-experimental design was employed to examine the relationship between two variables. The study population included all patients clinically diagnosed as suspected tuberculosis cases, encompassing pediatric and adult patients. A total of 40 fresh urine samples were collected and analyzed for this study.

TB detection via GeneXpert

Molecular rapid testing was conducted under biosafety conditions, utilizing appropriate personal protective equipment. The GeneXpert System, integrated with GX2.1 software (Cepheid Inc., Sunnyvale, USA) and single-use Xpert MTB/RIF cartridges (Cepheid Inc., Sunnyvale, USA), were employed for the analysis. Urine samples were initially transferred into sterile tubes and centrifuged at 3000 rpm for 15 minutes. The supernatant was carefully discarded into a disinfectant-containing container. The pellet was resuspended with the reagent to a final volume of 2 mL. The mixture was then thoroughly homogenized and allowed to stand for 15 minutes to minimize aerosol formation. The prepared sample was slowly pipetted into the GeneXpert cartridge, ensuring no bubbles were introduced. The cartridge was then securely closed and inserted into the GeneXpert module. Results were obtained in approximately 2 hours.

LAM testing

LAM testing was performed using the Abbott Determine TB LAM Ag Kit (Abbott Diagnostic Scarborough, USA). A 60 µL aliquot of the urine sample was pipetted onto the LAM test strip, with results interpreted within 25 minutes. The presence of a purple line indicated a positive result, while the absence indicated a negative outcome.

Data analysis

The test results were compiled into a 2×2 contingency table, and a receiver operating characteristic (ROC) curve was generated to represent the trade-off between sensitivity and specificity visually. ROC curve analysis was performed using SPSS software to assess the diagnostic accuracy of the LAM test, with the area under the curve (AUC) used as a measure of test performance. An AUC value approaching 1.0 signifies a highly accurate diagnostic test. Sensitivity and specificity were calculated using the formulas outlined by Sastroasmoro & Ismael (2011):

$$\text{Sensitivitas} = \frac{a}{a+c} \times 100\%$$

$$\text{Spesifitas} = \frac{d}{b+d} \times 100\%$$

Where:
a : samples with both GeneXpert (+) and LAM (+)
b : samples with GeneXpert (-) and LAM (+)
c : samples with GeneXpert (+) and LAM (-)
d : samples with GeneXpert (-) and LAM (-)

Results

The distribution and frequency data for GeneXpert and LAM test results are presented in **Table 1**. GeneXpert testing yielded negative results in 34 out of 40 samples (85%) and positive results (Rifampicin sensitive/ resistant) in 6 out of 40 samples (15%). The LAM test showed negative results in 28 out of 40 samples (67.5%) and positive results in 13 out of 40 samples (32.5%). The calculated sensitivity was 100%, and specificity was 79.4%. A sensitivity of 100% indicates that all patients with the disease were correctly identified as positive by the test. Conversely, the specificity value suggests that the LAM test accurately identified 79.4% of individuals without the disease as a true-negative for MTB, with the remaining 20.6% having a possibility of false-positive results (positive predictive value) for MTB.

The relationship between sensitivity and specificity was graphically represented using an ROC curve, with sensitivity on the y-axis and specificity on the x-axis (**Figure 1**). The study's ROC curve is shown by the blue line above the red diagonal line representing the positive cases. The lack of overlap between the blue and red lines indicates that the study has an ideal separation metric for distinguishing between positive and negative cases perfectly. The ROC curve result is supported by an AUC value of 0.897 (asymptotic significance 0.002), which is classified as good within the range of 0.80–0.90 (Gorunescu, 2011).

Discussion

A positive GeneXpert result cannot distinguish between active and dead MTB, so positive GeneXpert results should be confirmed with sputum culture. A negative GeneXpert result in this study does not rule out the possibility of latent TB, a condition where MTB is present in the body but remains inactive and does not cause symptoms (Kiazyk & Ball, 2017). Latent TB can be confirmed with a tuberculin skin test (TST) or interferon-γ release assays (Muñoz *et al.*, 2015).

This study found a sensitivity of 100% and a specificity of 79.4%, with the study population including both pulmonary and extrapulmonary TB patients, without knowing their HIV status. These findings support the sensitivity and specificity values of the LAM test reported in previous studies. Paris *et al.* (2017) reported LAM test sensitivity of 95% and specificity of 80% in TB patients with HIV-negative status. In the researh by Songkhla *et al.* (2019), the LAM test demonstrated a sensitivity of 75.0% and specificity of 76.0%, increasing sensitivity in HIV-infected patients with CD4 counts <50/µL. Previous LAM studies have predominantly used samples from suspected TB-HIV patients with CD4 counts <100/µL, showing higher sensitivity in TB-HIV patients compared to those without HIV infection (Dheda *et al.* 2010). Despite the moderate specificity of the LAM test in this research, the possibility of false-positive results should still be considered.

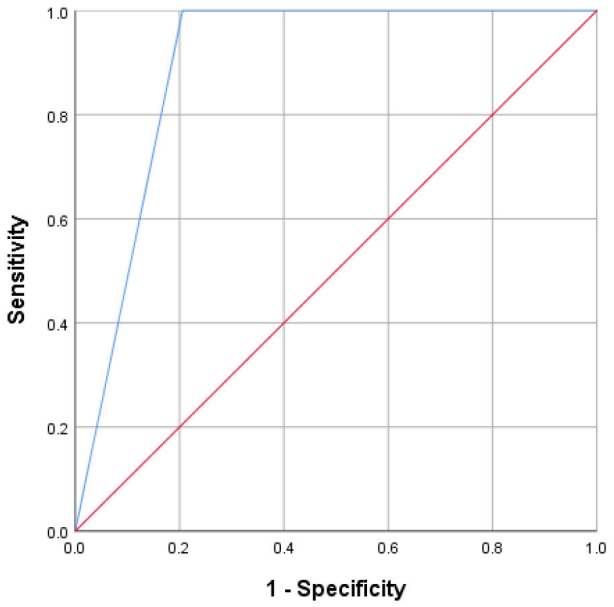

Figure 1 ROC curve with sensitivity on the y-axis and specificity on the x-axis. The blue line represents the study's ROC curve, which lies above the red diagonal line representing positive cases. The absence of overlap between the blue and red lines indicates an ideal separation metric for perfectly distinguishing between positive and negative cases.

Table 1 Sensitivity and specificity of the lipoarabinomannan (LAM) test compared to GeneXpert

Test		GeneXpert			Sensitivity* (%)	Specificity** (%)
		Positive	Negative	Total		
LAM	Positive	6	7	13	6/6 (100)	27/34 (79.4)
	Negative	0	27	27		
Total		6	34	40		

*Sensitivity: samples with GeneXpert (+) and LAM (+) divided by the total number of GeneXpert positive samples. **Specificity: samples with GeneXpert (-) and LAM (-) divided by the total number of GeneXpert negative samples.

Early diagnosis of pulmonary TB using the GeneXpert rapid molecular test facilitates earlier and more appropriate treatment. This test can be used for sputum, urine and stool samples while still maintaining high validity. Rapid molecular testing is highly recommended in hospitals that can reach remote areas with high TB cases and dense populations (Sahiratmadja *et al.*, 2020). However, urine-based LAM testing is not yet a government program, so it is only available at the patient's expense. Nevertheless, the LAM test has advantages such as accessible sample collection, rapid results, no need for specific equipment, or specialized expertise.

Conclusion

Urine-based LAM testing in suspected TB patients showed a high sensitivity of 100% and moderate specificity of 79.4% compared to GeneXpert.

Acknowledgments The authors would like to thank all the laboratory staff at the Sidawangi Lung Hospital in West Java Province for their assistance in this research.

Funding DI received funding in 2023 through the Study Assignment Program of the Ministry of Health of the Republic of Indonesia.

Conflict of interest All authors declare no conflicts of interest related to this research.

Author contributions DI wrote the manuscript and conducted the research; ZR designed the study; AC analyzed the research data; and FM interpreted the research data.

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