



Comparative Diagnostic Accuracy of LF-LAM TB Antigen and Xpert MTB/RIF in Pulmonary Tuberculosis among Underweight Patients

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Abstract

Background: In 2023, the global incidence of tuberculosis (TB) reached 8.2 million cases. It is the highest on record due to delayed diagnoses and a rising number of TB patients. Tuberculosis is more prevalent among patients with compromised immune systems, including those with HIV and malnutrition (BMI <18.5 kg/m²), who exhibit increased vulnerability to infection. The challenge of sputum expulsion impedes diagnosis, requiring a rapid, cost-effective early-detection technique. The lateral flow lipoarabinomannan TB antigen (LF-LAM TB-Ag) assay provides an alternate method for identifying lipoarabinomannan in urine, a constituent of the *Mycobacterium tuberculosis* cell wall. This study assessed the effectiveness of the LF-LAM TB-Ag assay compared with the Xpert MTB/RIF assay for TB diagnosis.

Methods: A comparative cross-sectional study was conducted at Abdul Moeloek Hospital, Lampung Province, from January 2023 to June 2024. A total of 52 suspected pulmonary TB patients with HIV-negative status and underweight BMI were evaluated using both the LF-LAM TB-Ag and Xpert MTB/RIF assays.

Results: Chi-square tests were used to compare the performance of LF-LAM TB-Ag with Xpert MTB/RIF. The LF-LAM TB-Ag test had a sensitivity of 79.59% and a specificity of 100% ($P=0.002$).

Conclusion: This study underscores the necessity for early detection of pulmonary TB in underweight individuals. The integration of LF-LAM TB-Ag with Xpert MTB/RIF improves detection, especially in high-risk populations, enabling prompt treatment and enhanced disease management.

Keywords: diagnostic, LF-LAM TB-Ag, screening, tuberculosis, Xpert MTB/Rif

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INTRODUCTION

Tuberculosis (TB) is a significant worldwide health issue and a primary cause of morbidity. The World Health Organization (WHO) Global TB Report 2024 indicates that there were 8.2 million new TB cases globally in 2023, an increase from 7.5 million cases in 2022. The increase in cases indicates delays in TB diagnosis, partially attributable to the effects of the COVID-19 pandemic, alongside a general rise in disease incidence.¹

Indonesia presently holds the second-highest prevalence of TB worldwide, trailing only India. Multiple reasons, such as elevated population density, restricted access to healthcare facilities, and the evolution of drug-resistant strains, contribute to the extensive incidence of TB in the country. In 2023, Indonesia was expected to have 1,060,000 new TB cases (385 per 100,000 individuals) and an annual TB-related mortality rate of 144,000, which includes instances of TB co-infection with human immunodeficiency virus (TB-HIV).¹

In Lampung Province, the TB case detection rate (CDR) is a mere 57%, considerably lower than the Indonesian government's objective of 90%. The poor detection rate signifies that a considerable proportion of TB cases are undiagnosed and untreated, facilitating ongoing TB transmission in the region.²

Pulmonary TB is widespread among underweight individuals, as starvation compromises the immune system, increasing susceptibility to *Mycobacterium tuberculosis* (MTB) infection. Inadequate nutritional status hinders the body's capacity to initiate a robust immunological response, hence elevating the risk of TB progression and severity. Conversely, pulmonary TB frequently results in considerable weight loss attributable to heightened metabolic demand, diminished appetite, and systemic inflammation.^{3–5}

The vicious cycle of malnutrition and TB can worsen disease outcomes, prolong recovery, and increase mortality risk, highlighting the importance of nutritional support in TB management. Underweight status is commonly defined by a body mass index (BMI) <18.5 kg/m².⁶ It is associated with increased susceptibility to TB and altered disease progression, necessitating tailored diagnostic approaches.^{3,6}

The Xpert MTB/RIF test is highly efficient in identifying and diagnosing TB from sputum samples, providing superior accuracy compared to acid-fast bacilli (AFB) microscopy. It has a sensitivity of 99% and a specificity of 98%, compared with 60% and 95% for AFB, respectively. With a rapid turnaround time of 2–3 hours, it serves as a dependable instrument for diagnosing pulmonary TB. The test necessitates high-quality sputum specimens (mucopurulent or purulent) of a minimum volume of 3–5 cc.^{7–9}

The microbiological diagnosis of pulmonary TB is typically performed using sputum-based tests, including AFB microscopy and Xpert MTB/RIF molecular testing. However, these methods have limitations, as some patients struggle to produce sputum, particularly those who are malnourished or have advanced HIV. Furthermore, sputum-based

tests cannot detect extrapulmonary TB unless there is lung involvement. Therefore, there is research into alternative diagnostic approaches, such as urine-based TB detection.^{10,11}

Urine-based TB testing offers several benefits, including a less invasive collection process, simpler laboratory requirements, and lower infection risk for healthcare workers. Moreover, it can identify extrapulmonary TB by detecting circulating MTB antigens. This method specifically targets LAM antigens, which are essential components of the mycobacterial cell wall.^{10,12}

The Lateral Flow Lipoarabinomannan TB Antigen (LF-LAM TB-Ag) test can be used as a diagnostic instrument for the early identification of MTB presence. This test kit is less invasive than sputum specimens, with a simpler examination room setup, poses a lower risk of exposure and infection to examiners, and can identify extrapulmonary tuberculosis via MTB in human urine.^{13,14}

The WHO initially endorsed the LF-LAM TB-Ag as an early diagnostic tool for TB in HIV-positive individuals in 2015, with an updated recommendation released in 2019. Nonetheless, its application is constrained by its inadequate sensitivity and specificity, as evidenced by multiple investigations. Research indicates that LF-LAM TB-Ag is not particularly efficient in identifying TB in people without HIV co-infection, severe clinical conditions, or other immunocompromised states.^{13,14}

Tessema et al found that LF-LAM TB-Ag testing could assist in diagnosing undernourished TB, with a sensitivity of 65.4% and specificity of 82.9%. This low-cost test requires just 60 µl of urine and provides results within 25 minutes. Due to its simplicity and efficiency, LF-LAM TB-Ag is a valuable screening tool for severe TB cases, particularly in patients with HIV or malnutrition, making it a promising alternative for TB diagnosis.^{14,15}

This study aimed to evaluate the LF-LAM TB-Ag diagnostic test in comparison to Xpert MTB/RIF for detecting pulmonary TB in malnourished (underweight) individuals who are non-HIV TB suspects.

METHODS

This research used a cross-sectional diagnostic accuracy design. Primary data were collected through the observation of patients in inpatient wards at Abdul Moeloek Hospital, Lampung Province, from January 2023 to June 2024. The Institutional Review Board of the Research Ethics Committee of Abdul Moeloek Hospital, Lampung Province, accepted this study under approval number 010/KEPK-RSUDAM/VIII/2022. All participants furnished written informed consent, which was appropriately witnessed.

Suspected pulmonary TB patients with underweight BMI ($<18.5 \text{ kg/m}^2$) were evaluated using both the LF-LAM TB-Ag assay in urine and the Xpert MTB/RIF assay in sputum, and the diagnostic efficacy was analyzed. Initially, 70 patients were enrolled, but only 52 were included in the final analysis after applying the predefined inclusion and exclusion criteria. Therefore, the sampling technique was used total sampling, including all 52 eligible underweight patients who met the inclusion criteria.

The study involved newly suspected cases of pulmonary TB in patients who were able to expectorate sputum. To eliminate potential confounding factors, patients with chronic conditions such as diabetes mellitus, hepatitis, chronic renal failure, malignancy, or HIV were excluded.

This test collects a midstream urine sample from the patient in the morning using a sterile urine tube. The test should be conducted within 30–60 minutes of collection. If stored at room temperature, the LF-LAM TB-Ag test can be performed within 8 hours, and if refrigerated at $2-8^\circ\text{C}$, it remains valid for up to 3 days.

To begin the test, open the Alere Determine LAM TB-Ag kit by removing the front foil seal from the top. Label the kit with the patient's identification code. Using a micropipette, dispense $60 \mu\text{l}$ of urine onto the sample pad of the test kit. Allow 25 minutes for the reaction to occur. Results should be read under normal room lighting within 25 minutes, but no later than 35 minutes to ensure accuracy.

The Xpert MTB/RIF assay is a rapid molecular test for detecting MTB and rifampicin resistance (RIF-R) using a sputum sample. The procedure begins by mixing the morning's sputum with a sample reagent in a 2:1 ratio, shaking it for 10–15 minutes to liquefy and inactivate the sample. The processed sample is then transferred into the Xpert MTB/RIF cartridge, which is placed in the GeneXpert machine. The system automatically performs DNA extraction, amplification, and real-time PCR analysis, providing results in approximately 2 hours.

The effectiveness of the LF-LAM TB-Ag assay was assessed by determining its sensitivity and specificity in comparison to the Xpert MTB/RIF assay. Data analysis was conducted using IBM SPSS version 21.0. The chi-square test was used to evaluate significant differences between the two diagnostic approaches at the 95% confidence level, with $P < 0.05$ considered statistically significant.

RESULTS

There were 52 subjects in this study, consisting of 14 subjects (26.9%) above 60 years, 17 subjects (32.7%) in the range of 44–60 years, 20 subjects (38.5%) in the range of 19–43 years, and 1 subject (1.9%) in the 10–19 years age range. Among suspected pulmonary TB patients with HIV-negative status and low BMI (underweight), 42 subjects were male and 10 subjects were female. All patients were newly diagnosed with TB (Table 1).

Table 1. Characteristics of research subject (n=52)

Variables	n	%
Age (years)		
Above 60	14	26.9
>44–60	17	32.7
>19–44	20	38.5
>10–19	1	1.9
Gender		
Male	42	80.8
Female	10	19.2
Low Body Mass Index (BMI)		
Severe ($<17.0 \text{ kg/m}^2$)	30	57.7
Mild ($17.0-18.5 \text{ kg/m}^2$)	22	42.3

A bivariate analysis was performed to evaluate the effectiveness of the LF-LAM TB-Ag test compared with Xpert MTB/RIF in suspected TB

patients with malnutrition (mild to severe underweight). The analysis was conducted using a 2x2 contingency table (Table 2), which determined that the LF-LAM TB-Ag test had a sensitivity of 79.59% and a specificity of 100%.

Table 2. Performance of LF-LAM TB-Ag as compared to Xpert MTB/Rif among TB malnourished patients

LF-Lam TB-Ag	Xpert MTB/Rif		P
	Positive	Negative	
Positive	39 (a)	0 (b)	0.002
Negative	10 (c)	3 (d)	

Note: a=True Positive (TP); b=False Positive (FP); c=False Negative (FN); d=True Negative (TN)

$$\text{Sensitivity} = \frac{a}{(a+c)} \times 100\% = \frac{39}{49} \times 100\% = 79.59\%$$

$$\text{Specificity} = \frac{d}{(b+d)} \times 100\% = \frac{3}{3} \times 100\% = 100.00\%$$

Comparative test data between LF-LAM TB-Ag and Xpert TB/Rif were conducted using chi-square and obtained a significance value of $P=0.002$, as shown in Table 2, which indicates the performance of LF-LAM TB-Ag compared to Xpert MTB/Rif in determining the diagnosis of TB patients.

Underweight status was evaluated as a potential risk factor for increased susceptibility to pulmonary TB infection. Patients were categorized as mildly or severely underweight based on Southeast Asian BMI criteria. Subgroup analysis showed that the LF-LAM TB-Ag test using urine specimens was more commonly found in severely underweight individuals (80.00%) compared to those with mild underweight (68.1%). The sputum specimens were tested using Xpert MTB/RIF in the same subgroups and found that similar in severely underweight and mildly underweight individuals, namely 93.33% and 95.45%, respectively, as shown in Table 3.

Table 3. Performance of LF-LAM TB-Ag in detecting TB among malnourished patients

Indicators	Body Mass Index	
	Severe underweight	Mild underweight
LF-Lam TB-Ag		
Positive	24 (80.00%)	15 (68.18%)
Negative	6 (20.00%)	7 (31.82%)
Xpert MTB/Rif		
Positive	28 (93.33%)	21 (95.45%)
Negative	2 (6.67%)	1 (4.45%)

DISCUSSION

This study found that the majority of respondents were males aged between 19 and 44 years. This is consistent with the study by Marcoa et al, which reported that the highest prevalence of pulmonary TB occurred after the second decade of life, specifically in the 20–59 age range, with males comprising 65.8% of cases. Males tend to have more comorbidities and risk factors for pulmonary TB, including higher rates of smoking, alcohol consumption, and extramarital sexual behavior across all age groups, as well as a greater likelihood of HIV co-infection.¹⁶

A study by Victoria Peer et al revealed that the incidence of pulmonary TB was significantly higher in men than in women, with an incidence rate ratio (IRR) ranging from 1.25 to 1.81. This indicates that men are 1.25 to 1.81 times more likely to develop pulmonary TB compared to women. This disparity may be attributed to biological, behavioral, and social factors, including differences in immune response, occupational exposure, and healthcare-seeking behavior between men and women.¹⁷

In a recent study, most respondents suspected of having pulmonary TB with a low BMI were classified as severely underweight ($<17 \text{ kg/m}^2$) and mildly underweight ($17\text{--}18.5 \text{ kg/m}^2$), with 30 individuals (57.7%) and 22 individuals (42.3%), respectively. Su Hwan Cho et al conducted a cohort study in 2022 and found that mildly underweight and severely underweight individuals had a 2.0-times and 2.8-times increased risk of developing TB, respectively.¹⁸ Underweight is considered an indicator of malnutrition in adults, although this anthropometric measure does not fully reflect all nutritional deficiencies.¹⁸

This study obtained that the sensitivity of the LF-LAM TB-Ag test compared to Xpert MTB/RIF was 79.59%, with a specificity of 100% ($P=0.002$) for diagnosing pulmonary TB in HIV-negative malnourished (underweight) patients. LF-LAM TB-Ag was found more in pulmonary TB in severely underweight individuals (80.0%) compared to those who were mildly underweight (68.1%). In contrast,

the LF-LAM TB-Ag urine assay in the same groups demonstrated was higher than the Xpert MTB/RIF assay in both severely underweight (93.33%) and mildly underweight patients (95.45%). This indicates that although the performance of LF-LAM TB-Ag to detect pulmonary TB in individuals with a low BMI is still lower than the Xpert MTB/RIF test, its sensitivity shows an increase when used in suspected TB cases with severe underweight rather than mild underweight (80.00% vs. 68.18%).

This study aligns with the findings of Elhalawany et al in Egypt, who reported that urinary LF-LAM TB-Ag demonstrated a high sensitivity of 95.7% and specificity of 98.1%, with a positive predictive value (PPV) of 95.7% and a negative predictive value (NPV) of 98.1%, achieving an overall accuracy of 97.4% in detecting TB among HIV co-infected patients. LF-LAM TB-Ag concentrations were strongly correlated with low CD4 counts, deteriorating nutritional status, and significant radiological abnormalities.¹⁹

Tlali et al in South Africa conducted an LF-LAM TB-Ag test in TB patients with advanced HIV. Among 105 patients with a positive sputum culture for *Mycobacterium tuberculosis*, it was observed that LF-LAM TB-Ag sensitivity was higher in patients with lower BMI compared to those with a normal BMI, which were 52.6% and 35.8%, respectively.²⁰ Ogundeji et al in Nigeria, also stated that the LF-LAM test had a low sensitivity of 57.5% but a specificity of 100% in TB and HIV co-infected patients.²¹

Pulmonary TB develops when MTB bacilli reach the alveoli and are phagocytosed by alveolar macrophages. In severely malnourished individuals, impaired cellular immunity fails to contain bacterial growth, resulting in proliferation and hematogenous dissemination. During active infection, MTB releases LAM, a glycolipid component of its cell wall, into the bloodstream.¹⁰

Micronutrient deficiencies, particularly zinc, vitamin D, vitamin A, and selenium, further impair macrophage and T-cell function, reducing bactericidal activity, cathelicidin synthesis, and overall immune defense, which facilitates reactivation from latent to active TB.²² Severe

underweight is characterized by reductions in CD4⁺ and CD8⁺ lymphocytes, leading to defective granuloma formation and caseous necrosis. This breakdown permits MTB dissemination, increasing bacterial burden and LAM release into circulation.^{15,23,24}

Additionally, malnutrition is often accompanied by hypoalbuminemia, which can increase the permeability of renal blood vessels and cause the leakage of LAM molecules into the urine. In cases of severe underweight, patients are also more susceptible to more severe or disseminated TB, further elevating LAM levels in the blood and their excretion through the kidneys due to the combined effects of malnutrition, immune suppression, and increased bacterial load.^{15,23,24}

Research on the efficacy of LF-LAM TB-Ag as a diagnostic tool for detecting pulmonary TB in non-HIV patients with pulmonary TB is still very limited. As of 2019, the WHO recommends the use of the LF-LAM TB-Ag test only for TB-HIV co-infected patients with a CD4 count <100 cells/ μ L or those with severe clinical symptoms without prior CD4 assessment. This recommendation is due to the sensitivity and specificity of LF-LAM TB-Ag, as reported in many previous studies, still unsatisfactory.^{25,26}

The study underscores the distinct diagnostic strengths and limitations of the LF-LAM TB-Ag and Xpert MTB/Rif assays in detecting pulmonary TB among underweight patients. While the LF-LAM TB-Ag assay demonstrated moderate sensitivity (79.59%), particularly in patients with advanced disease or severe underweight status, its reduced sensitivity in early-stage TB and lower predictive values limit its utility as a standalone diagnostic tool.

Conversely, the Xpert MTB/Rif assay exhibited consistently high sensitivity across all BMI categories, highlighting its superior diagnostic accuracy and reliability in this vulnerable population. These findings suggest that although severe underweight status enhances the sensitivity of the LF-LAM TB-Ag assay, it compromises its predictive values, thereby limiting its utility as a standalone diagnostic tool.

LIMITATION

One major limitation of this study is the inability to include a large and diverse sample size in a multi-center setting. A larger sample would facilitate more comprehensive subgroup analyses, allowing for better comparisons between different patient groups. Underweight (BMI <18.5 kg/m²) is considered an indicator of malnutrition in adults. However, this anthropometric measure does not fully reflect all nutritional deficiencies, and this study didn't collect and analyze the albumin serum data. The LF-LAM TB-Ag urinary test is not exclusive to *Mycobacterium tuberculosis* and may yield positive results for other *Mycobacterium* species, such as *M. bovis*, *M. leprae*, and *M. avium*. Therefore, future studies are necessary to compare the sputum culture as a gold standard for diagnosing pulmonary TB to enhance test accuracy and improve research outcomes.

CONCLUSION

The LF-LAM TB-Ag assay, which reflects the pathophysiological alterations associated with malnutrition, complements the molecular accuracy of the Xpert MTB/RIF assay across different stages of disease. In accordance with WHO recommendations, LF-LAM should not replace Xpert MTB/Rif or sputum culture, but rather function as an adjunctive tool to strengthen early diagnostic capacity, particularly in high-risk or resource-limited settings. Integrating both assays may enhance diagnostic accuracy, facilitate earlier treatment initiation, and ultimately improve TB control outcomes.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article. There

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