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Research Article

Performance Evaluation of TB-LAMP in the Diagnosis of Pulmonary Tuberculosis

Teeba Alaa Hussein[1, *], Ali Muhsin Ali[2], Hussein Hameed Rahem[3]

[1]Department of Biology, Mustansiriyah University, Baghdad, Iraq
[2]Department of Biology, Baghdad University, Baghdad, Iraq
[3]National TB Institute, Ministry of Health, Baghdad, Iraq

Abstract

Background: Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB), is one of the most common infectious diseases, especially in countries with limited healthcare resources. Early and accurate diagnosis is essential to curb its spread, and loop-mediated isothermal amplification (LAMP) is a rapid, cost-effective, and reliable molecular diagnostic method. Objective: The objective of this study was to evaluate the performance of TB-LAMP as a rapid and reliable diagnostic tool for pulmonary TB and compare it with direct smears and Gene Xpert, using culture as a reference. Materials: Between February and July 2025, a total of 397 patients (227 men and 170 women) were enrolled in the study. Samples were tested by smear microscopy, culture, Xpert MTB/RIF, and TB-LAMP. Results: Of the 397 patients, 84 (21.15%) were culture-positive. Among culture-positive patients, 81 (96.4%) were positive by Gene Xpert, 69 (82.1%) by TB-LAMP, and 49 (58.3%) by direct smear microscopy. Using culture as the reference standard, the specificity, positive predictive value (PPV), and negative predictive value (NPV) of the TB-LAMP assay were 82.1% (73.9%–90.3%), 99.0% (97.9%–100%), 95.8% (91.2%–100%), and 95.4% (93.1%–97.7%), respectively. Conclusions: The TB-LAMP assay exhibits high diagnostic accuracy for the detection of *Mycobacterium tuberculosis*, making it a rapid, cost-effective, and reliable alternative to conventional methods, especially in resource-poor settings.

Keywords

Tuberculosis (TB), Mycobacterium Tuberculosis (MTB), Gene Xpert, TB-LAMP

1. Introduction

Tuberculosis (TB) is severe infectious disease caused by infection with *M. tuberculosis* (MTB). TB is still one of the most pressing health problems worldwide, particularly in rural and impoverished countries [1]. Correct diagnostic tools are essential for TB control and the initiation of treatment. TB-LAMP is an affordable, rapid molecular test [2]. *Mycobacteria* are rod-shaped (bacilli), non-motile organisms, typically 0.2–0.6 µm in diameter and 1.0–10 µm in length by electron micrograph comparison although some species, such as *Mycobacterium leprae,* can be far larger, clinically up to 100 µm in length. They are characterized by an unusual cell wall with mycolic acid, which provides resistance against an adverse environment [3]. *M. tuberculosis* (MTB) is the pathogenic mycobacterium in humans; it preferentially infects the lungs, resulting

*Correspondence: Teeba Alaa Hussein (tebaalaa@uomustansiriyah.edu.iq)

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in TB. Multidrug-resistant (MDR) *M. tuberculosis* is an important clinical problem as it causes life-threatening infections and drug resistant form of the disease [4]. MTB has no excretion of toxins or classical virulence factors. Its pathogenicity is resistance to macrophages that leads to a Granuloma [5]. The primary routine diagnosis for *M. tuberculosis* includes direct smear microscopy, culture, and molecular tests like GeneXpert. Smear microscopy is an old, simple, rapid, and cost effective method, but less sensitive, particularly in samples having lower bacillary load [6]. Advanced molecular tests such as Gene-Xpert and line probe assay (LPA) show high sensitivity and specificity for the diagnosis MTB, but are costly and technically demanding [7]. On the other hand, culture in Lowenstein-Janssen (L-J) medium is the reference method for tuberculosis diagnosis, and it is highly specific and sensitive; unfortunately time required to obtain results can be very long, up to 8 weeks [8]. An imperative requirement is the development of a fast, inexpensive, and sensitive diagnostic test for MTB. Recently, the World Health Organization (WHO) recommended an alternative molecular test for *M. tuberculosis*, termed loop-mediated isothermal amplification (LAMP), instead of smear microscopy in peripheral laboratories. This is a novel RT-PCR test by Eiken Chemical Co., Ltd. [9]. TB-LAMP is an inexpensive, fast, easy to use, and very sensitive diagnostic method. While GeneXpert technology is based on PCR and multiple cycles, TB-LAMP integrates LAMP to amplify defined DNA targets in (*gyrB* and *IS*) regions of MTB. It involves six regions of the target gene (B1, B2 and B3 at 5′ and F3, F2 and F1 at 3′ of the target) by using four specially designed primers previously described [16], with strand-displacing polymerase (DNA polymerase) used to make large amounts of DNA from minuscule quantities of target nucleic acids at a constant temperature (65 ℃). Results are based on observing turbidity or fluorescence that is apparent to the naked eye under an ultraviolet light in less than an hour. Results can be obtained in less than 2 hours [10]. It had been reported in many reports that our developed TB-LAMP is a rapid and suitable approach to diagnose MTB [11-13]. We aimed to assess the role of TB-LAMP as a diagnostic tool for *Mycobacterium tuberculosis* and to compare it against direct smear and Gene Xpert. Culture was the gold standard for analysis of clinical sputum samples from TB patients.

2. Materials and Methods

2.1. Study Design

An original study was conducted at the National Institute for Tuberculosis /National Reference Laboratory, in Baghdad, over a period of seven months from February to July 2025. Patients with clinical signs of TB (fever, weight loss, night sweats) were enrolled.

2.2. Sample Size

A total of 397 patients were enrolled in this study. The age and gender of each patient were recorded.

2.3. Sample Collection

Three sputum samples were collected from each patient at different times (morning, evening, and at the health center) and pooled for testing. Samples were processed for smear microscopy, culture, Gene Xpert, and TB-LAMP to evaluate their diagnostic performance compared to reference standards.

2.4. TB-LAMP

The TB-LAMP assay was carried out using the Loopamp MTBc kit (Eiken, Japan) according to the manufacturer’s instructions, with minor modifications for sputum samples. Initially, 60 µl of sputum was placed in a heating tube and incubated at 90°C for 5 minutes to inactivate pathogens. After cooling at room temperature for 2 minutes, the tube contents were transferred to an absorbent tube and shaken vigorously to mix thoroughly. Approximately 30 µl of the treated sputum was then moved to a reaction tube and incubated in a turbidimeter at 65°C for 40 minutes. The amplification results were assessed visually: positive samples showed fluorescence observable under ultraviolet light, whereas negative samples remained non-fluorescent [14].

2.5. Gene Xpert

The Gene Xpert assay (Cepheid, USA) was performed according to the manufacturer's instructions. Briefly, two volumes of buffer were added to the sputum sample, vortexed thoroughly, and incubated at room temperature for 15 minutes. The prepared sample was then transferred to the kit and loaded into the Gene Xpert device for automated analysis [15].

2.6. Culture and Direct Smear

The sputum samples were processed using a modified Petroff method, and inoculated onto Löwenstein-Jensen (L-J) medium. Each sample was first mixed with an equal volume of NaOH, vortexed thoroughly, and left at room temperature for 15 minutes to decontaminate. The total volume was then adjusted to 50 ml with phosphate-buffered and centrifuged at 4000 rpm for 15 minutes. After discarding the supernatant, the pellet was resuspended by vortexing.

From the resuspended pellet, (200) µl was inoculated onto L-J medium and incubated at 35°C for 6 to 8 weeks. In parallel, smears were prepared from the pellet, stained using the Ziehl–Neelsen (ZN) method, and examined under a light microscope for acid-fast bacilli [16].

2.7. Statistical Analysis

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for Gene Xpert, TB-LAMP,

and direct smear microscopy were calculated using SPSS version 22 (USA). The 95% confidence intervals (CI) for all parameters were estimated and cross-validated using Medcalc online software.

3. Results

Of the 397 patients, 227 (57.1%) were male, and 170 (42.8%) were female. Patient ages ranged from 16 to 60 years, with a median age of 39 years.

Of the 397 patients, 84 (21.15%) tested positive for *M. tuberculosis* culture (Figure 1). *M. tuberculosis* colonies are rough, tough, yellow-brown, and waxy and are positive on L-J culture medium. Among these patients with positive *M. tuberculosis* culture, 81 (96.4%) were tested using Gene Xpert technology, 69 (82.1%) using TB-LAMP technology, and 49 (58.3%) using direct smear microscopy (Figure 2). Positive TB-LAMP tests are characterized by visible fluorescence (Figure 3). For false-positive results in culture-negative patients, one patient is positive in the Gene Xpert test, while three patients are positive in the TB-LAMP test, and eighteen patients are positive using direct smear microscopy.

Figure 1. *Mycobacterium tuberculosis (MTB) colonies on Lowenstein-Jensen (L-J) media.*

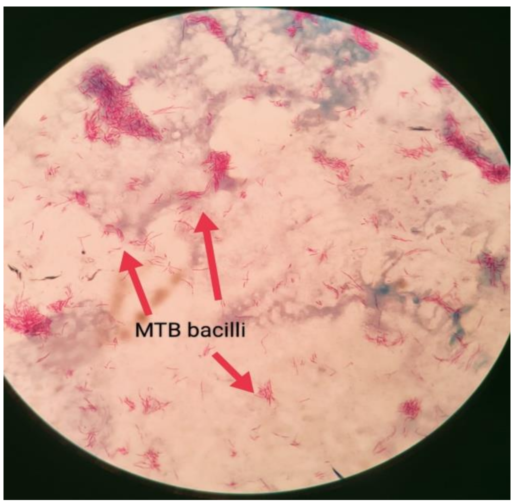

Figure 2. *MTB bacilli under light microscope.*

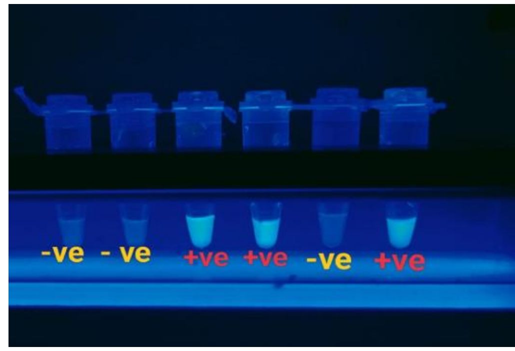

Figure 3. *Positive and Negative results of MTB by TB-LAMP test.*

Using culture as the reference standard, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of each diagnostic method, along with their 95% confidence intervals (CI), are summarized in Table 1.

Table 1. *Diagnostic performance of Gene Xpert, TB-LAMP, and Direct Smear microscopy compared with culture (95% CI).*

Test	Sensitivity% [95% CI]	Specificity% [95% CI]	PPV% [95% CI]	NPV% [95% CI]
Gene xpert	(92.4–100%) 96.4	(99.1–100%) 99.7	(96.4–100%) 98.8	(97.9–100%) 99.0
TB-LAMP	(73.9–90.3%) 82.1	(97.9–100%) 99.0	(91.2–100%) 95.8	(93.1–97.7%) 95.4
Direct smear	(47.8–68.8%) 58.3	(91.6–96.8%) 94.2	(62.5–83.7%) 73.1	(86.1–92.7%) 89.4

PPV= Positive predictive value, NPV= Negative predictive value, CI= Confidence Intervals.

The Receiver Operating Characteristic (ROC) curve analysis in this study demonstrated that Gene Xpert had the highest diagnostic accuracy with an Area Under the Curve (AUC) of 0.987, followed by TB-LAMP (AUC = 0.909) and smear microscopy (AUC = 0.762). These findings highlight the superior reliability of molecular methods compared to conventional smear microscopy (Figure 4).

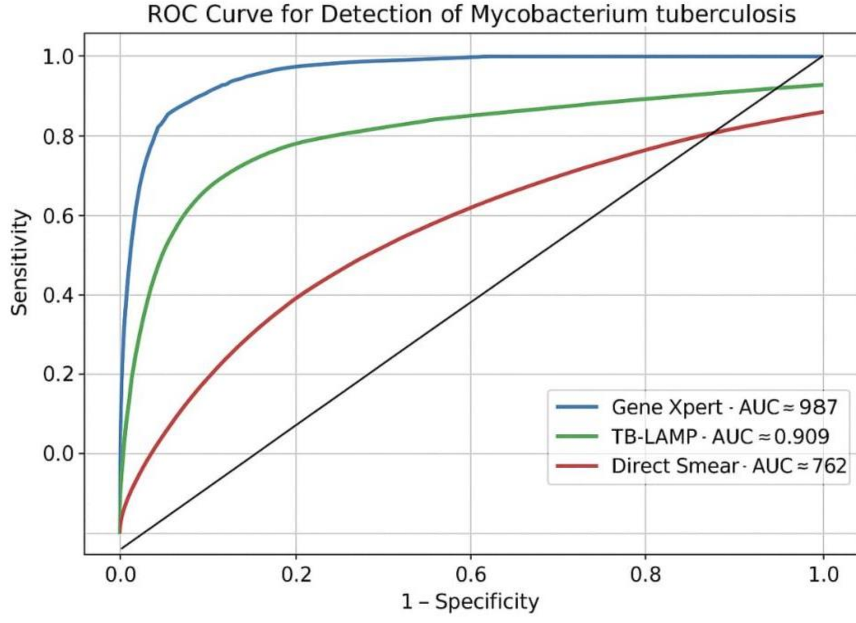

Figure 4. *The ROC curve analysis in this study Gene Xpert, TB-LAMP, and smear microscopy.*

4. Discussion

In low-resource settings, there is a critical need for rapid and accurate diagnostics to identify TB. Despite the high performance of Gene Xpert, its cost and infrastructure make generalisation difficult, as well as more affordable and simpler alternatives to molecular tests [17]. The study aim was to compare the performance and diagnostic accuracy of TB-LAMP with that of direct sputum smear microscopy and Gene Xpert, using culture as a gold standard in clinical sputum specimens for the detection of *M. tuberculosis* (MTB). This research demonstrated that the TB-LAMP is highly accurate for the diagnosis of pulmonary tuberculosis in sputum samples. The sensitivity of TB-LAMP was 82.1%, and the specificity was 99.0%, which is, higher confidence than that by the gold standard culture. Positive and negative predictive values of TB-LAMP were high (95.8% and 95.4%, respectively), demonstrating the relevance of TB-LAMP for confirming or excluding tuberculosis in suspected cases. These findings are also in agreement with previous observations. Yadav *et al.* reported equivalent sensitivity (82%), but a lower specificity (96.8%) [18], Gilao *et al.* demonstrated higher sensitivity and specificity than those of our study (92%) and higher specificity (97.2%) [19]. Our sensitivity was slightly lower than that of Gilao *et al.* which is likely due to dissimilar clinical sample quality, bacterial loads, or patient populations because of the lower sensitivity of TB-LAMP in the low bacteriopathy. The high specificity of TB-LAMP is especially critical in scenarios where false-positive testing could result in unnecessary treatment. In contrast with smear microscopy, where it is possible to observe *nontuberculous Mycobacteria* (NTM) acid-fast bacilli. TB-LAMP is designed for DNA sequences that are unique to *M. tuberculosis*, thereby reducing the likelihood of false-positive results. In our study, GeneXpert showed higher sensitivity (96.4%) than TB-LAMP. This is consistent with the results of multiple studies showing that GeneXpert has a sensitivity of over 95% in culture-confirmed TB cases. However, the specificity of GeneXpert may vary, as reported by Arora *et al.* (93.6%) and Rimal *et al.* (96.61%) [20, 21], and this specificity may be affected by the local prevalence of NTM or technical factors. GeneXpert has high sensitivity but is expensive and instrument-dependent, which limits its use in resource-poor settings. TB-LAMP technology is simpler, more cost-effective, more suitable for use in peripheral laboratories and field settings, and supports rapid TB diagnosis in high-burden settings. In this study, the sensitivity of TB-LAMP (82.1%) was significantly higher than that of smear microscopy (58–68%) in our group and other studies (e.g., Weldemhret *et al.* (68.38%) [22]. Smear microscopy is simple and rapid, but has low sensitivity and is particularly suitable for patients with low tuberculosis counts or HIV-positive patients, and may cross-react with NTM. The TB-LAMP test has an accuracy of 99%, is faster, and has higher sensitivity, making it an ideal method for screening smear-negative suspected TB cases [20, 22]. Comparative receiver operating characteristic (ROC) curve analysis confirmed that Gene Xpert was the most reliable diagnostic method for *Mycobacterium tuberculosis* (AUC = 0.987), followed by TB-LAMP (AUC = 0.909), and finally smear microscopy (AUC = 0.762). These results are consistent with previous studies [12, 23, 24] and emphasize the critical role of molecular testing in improving TB diagnosis. Although Gene Xpert has the highest diagnostic accuracy, TB-LAMP demonstrates comparable performance at a lower cost and with a simpler infrastructure, making it particularly suitable for decentralized laboratories. Smear microscopy,

while widely used, should not be used alone due to its limited diagnostic value.

5. Conclusion

TB-LAMP demonstrates superior diagnostic performance for tuberculosis testing. It is a rapid, cost-effective, and highly accurate alternative to Gene Xpert, particularly suitable for use in resource-limited settings. While Gene Xpert remains the most accurate method, TB-LAMP strikes a balance between accuracy, speed, and affordability. Its performance surpasses traditional microscopy and enables earlier diagnosis and treatment.

Abbreviations

AUC	Area Under Curve
CI	Confidence Intervals
LPA	Line Probe Assay
TB-LAMP	Loop-mediated Isothermal Amplification
L-J	Lowenstein-Janssen Medium
MDR-MTB	Multidrug-Resistant M. Tuberculosis
MTB	Mycobacterium Tuberculosis
NPV	Negative Predictive Value
NTM	Nontuberculous Mycobacteria
PPV	Positive Predictive Value
ROC	Receiver Operating Characteristic
TB	Tuberculosis
WHO	World Health Organization

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Author Contributions

Teeba Alaa Hussein: Formal Analysis, Investigation, Writing – original draft, Writing – review & editing

Ali Muhsin Ali: Formal Analysis, Conceptualization, Methodology, Project administration

Hussein Hameed Rahem: Conceptualization, Data curation, Resources, Supervision

Data Availability Statement

All data generated or analyzed in this study are included in this published paper (and its Supplementary Material files).

Conflicts of Interest

The authors declare no conflicts of interest.

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