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Comparative Diagnostic Accuracy of Genexpert, AFB Microscopy, LAMP and Symptom-Based Screening Against Culture in Pulmonary Tuberculosis

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Abstract:
Drug resistance and continued transmission of pulmonary tuberculosis (TB) caused by delayed diagnosis, which still a significant worldwide health concern. Mycobacterial culture is the gold standard for diagnosing tuberculosis, although takes a lot of time and resources. In order to assess their usefulness in government healthcare settings with limited resources. This study compares the diagnosis accuracy of four alternative techniques; GeneXpert, AFB microscopy, LAMP, and symptom-based screening vs against culture.
282 adult patients exhibiting pulmonary tuberculosis symptoms participated in a cross-sectional observational study. AFB microscopy, LAMP, symptom-based screening, GeneXpert, and culture (reference standard) were used to examine each subject. Sensitivity, specificity, receiver operating characteristic (ROC) curves, positive predictive value (PPV), and negative predictive value (NPV) were used to evaluate diagnostic accuracy.
Out of 282 subjects, 61.4% had TB confirmed by culture. Among the diagnostic methods, GeneXpert had the greatest positivity rate (76.2%), followed by symptom-based screening (88.3%), chest X-ray (70.3%), and AFB microscopy (69.0%). The positive rate for LAMP was the lowest at 39.7%. When compared to alternative techniques, GeneXpert showed acceptable specificity (66.0%) and better sensitivity (84.8%). Despite its high sensitivity (95.5%), symptom-based screening has a high false positive rate due to its low specificity (18.8%). LAMP exhibited the highest specificity (81.3%) but the lowest sensitivity (40.4%). The best diagnostic performance, according to ROC analysis, was GeneXpert (AUC: 0.801).
GeneXpert proved to be the most accurate and well-rounded diagnostic tool, especially in environments with inadequate infrastructure. Although symptom-based screening is helpful for preliminary triage, confirmation testing is necessary due to its low specificity.
Keywords: *Pulmonary tuberculosis, Diagnostic accuracy, GeneXpert MTB/RIF, Loop-mediated isothermal amplification (LAMP), AFB microscopy, Symptom-based screening*

1. INTRODUCTION

Tuberculosis (TB) is a major infectious disease worldwide, significantly impacting public health and socioeconomic stability. The WHO Global TB Report 2023 estimates that TB caused 1.25 million deaths and 10.8 million new infections globally, despite years of control efforts. India is heavily affected, accounting for nearly 28% of global TB cases, with about 2.8 million active cases each year. Approximately 40% of these patients receive timely and appropriate treatment, increasing the risk of transmission and the development of resistant strains. These trends emphasize the urgent need for early and accurate diagnosis to control TB effectively[1,2].

Diagnosing tuberculosis (TB) is difficult due to various clinical, systemic, and socioeconomic issues. In rural and resource-limited areas, access to advanced diagnostic tools like GeneXpert, Line Probe Assay (LPA), and Polymerase Chain Reaction (PCR) is limited, which usually delays the diagnosis. Many cases are delayed because of a lack of awareness among healthcare providers and the public, leading to misdiagnoses or late reporting. Additionally, the low bacterial count in many clinical samples makes it hard to obtain microbiological confirmation[3,4].

Traditional diagnostic methods such as symptom-based screening and sputum smear microscopy are prevalent in low-resource settings due to their simplicity and low cost. However, they have major

drawbacks that can hinder accurate diagnoses and effective treatment. Symptom-based screening correlates with bacteriological confirmation only before treatment initiation and becomes unreliable thereafter, especially in HIV co-infected patients. Sputum smear microscopy, while widely used, has low sensitivity in cases with low bacillary load and cannot distinguish viable organisms. Mycobacterial culture is still considered the gold standard, but it requires advanced facilities, is costly, and has a turnaround time of up to eight weeks, making it unsuitable for rapid clinical decision-making[5].

Recent advancements have introduced molecular diagnostic tools such as GeneXpert MTB/RIF, which allow rapid detection of Mycobacterium tuberculosis DNA and rifampicin resistance. However, these tests cannot confirm bacterial viability and often require invasive sampling, limiting their use in peripheral and pediatric settings. Stool-based GeneXpert testing has emerged as a promising alternative for children, but its routine implementation remains limited[4]. Loop-mediated Isothermal Amplification (LAMP) offers another molecular method with potential applicability in low-resource settings because it operates under isothermal conditions and does not require complex thermal cycling. Although improvements have been made to enhance LAMP performance, further evaluation is needed to confirm its accuracy and feasibility for large-scale use[6,7].

Differences in diagnostic practices between public and private healthcare sectors hinder tuberculosis (TB) control. Public facilities primarily use guideline-based microbiological testing, while private providers often rely on chest X-rays and clinical judgment, prioritizing convenience over accuracy. This leads to delays and diagnostic errors. To address these issues, it is essential to strengthen evidence-based diagnostic strategies and ensure the adoption of reliable, cost-effective tests across all healthcare systems[8].

On the purview of above facts, we hypothesize that disparity in diagnostic tools along with the resource constraints are the major cause of this substantial TB prevalence. Despite the availability of multiple diagnostic methods, there is limited evidence on their comparative performance under real-world programmatic conditions, especially in government healthcare settings with limited resources. Understanding the diagnostic accuracy of commonly used tools—GeneXpert MTB/RIF, AFB microscopy, LAMP, and symptom-based screening—against the gold standard of mycobacterial culture is essential to guide national TB programs in optimizing diagnostic algorithms. This study aims to address this gap by systematically comparing the sensitivity, specificity, predictive values, and ROC characteristics of these diagnostic methods in a high-burden setting. The findings are expected to provide practical insights for selecting the most effective diagnostic tools in resource-constrained environments and improving TB detection strategies.

2. METHODOLOGY

2.1. Study Design and Setting

The present study was a cross-sectional observational study conducted at the Civil Hospital, Palwal, Haryana, India. The study was carried out between June 2024 and January 2025. Ethical clearance was obtained from the Institutional Ethics Committee (IEC No.: NIMSUR/IEC/2022/315 dated 10/06/2022). Written informed consent was obtained from all participants before inclusion.

2.2. Sample Size Calculation

The sample size was calculated using G*Power software (version 3.1). Parameters were derived from effect size estimates reported in similar diagnostic accuracy studies. A power of 80%, alpha error of 0.05, and effect size of 0.3 were assumed. The minimum sample size required was 252 participants. Considering possible attrition, 282 participants were included in the study[9].

2.3. Participants

Adults aged 18 years and above with clinical suspicion of pulmonary tuberculosis were enrolled. Patients presenting with cough for more than two weeks, weight loss, night sweats, and fever were considered symptomatic. Individuals with extrapulmonary TB, pediatric cases, or those who refused consent were excluded.

2.4. Sample Collection and Processing

Two sputum samples (spot and early morning) were collected from each participant under standard infection control protocols. Samples were processed according to National TB Elimination Programme (NTEP) guidelines. Both samples were utilized for smear microscopy, GeneXpert MTB/RIF assay, LAMP assay, and culture.

2.5. Diagnostic Tests

- Symptom-based Screening: Participants were screened for four cardinal symptoms: persistent cough (>2 weeks), fever, weight loss, and night sweats. The presence of any two or more symptoms was considered

positive.

- AFB Microscopy: Direct smear examination was performed using the Ziehl–Neelsen staining technique. Two smears per patient were examined under oil immersion (100x objective).
- Mycobacterial Culture (Reference Standard): Sputum samples were inoculated on Lowenstein–Jensen (LJ) medium and incubated at 37°C. Cultures were observed weekly for up to 8 weeks for growth confirmation.
- GeneXpert MTB/RIF Assay: Testing was carried out using the GeneXpert system (Cepheid, USA). MTB/RIF cartridges (Lot 95292, Expiry Jan 2027) were used. For each sample, 2 mL of sputum was mixed with twice the volume of sample reagent (8 mL). The mixture was incubated at room temperature for 15 minutes and loaded into the cartridge for automated analysis.
- Loop-Mediated Isothermal Amplification (LAMP): LAMP testing was performed on the Compact XL platform (Mylab Discovery Solutions Pvt. Ltd., India). Standard isothermal amplification conditions recommended by the manufacturer were followed.
- Routine Laboratory Investigations:
  - Complete Blood Count (CBC): Conducted using the Cellenium® 380 Hematology Analyzer (Trivitron Healthcare, India).
  - Biochemical Parameters: Measured using the AutoChem™ Ingenious Clinical Chemistry Analyzer (ARKRAY Healthcare Pvt. Ltd., India).

2.6. Quality Control and Biosafety

All instruments were calibrated prior to use. Internal quality control was performed daily for hematology and biochemistry analyzers. Biosafety Level-2 practices were strictly followed during sample handling and culture procedures.

2.7. Statistical Analysis

Diagnostic accuracy of each test was calculated using mycobacterial culture as the gold standard. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were determined with 95% confidence intervals. Receiver Operating Characteristic (ROC) curves were plotted for each test, and Area Under Curve (AUC) values were compared. Logistic regression analysis was performed to identify predictors of TB positivity. All analyses were conducted using SPSS version 26.0 (IBM Corp., USA). A p-value <0.05 was considered statistically significant[10].

3. RESULTS

3.1. Participant Characteristics

A total of 282 participants were included in the analysis. There were no missing values for any variables evaluated, thus ensuring a robust analytical dataset.

3.2. Diagnostic Test Outcomes

Table 1 summarized the frequencies of various diagnostic parameters among the participants. Contrary to earlier studies where Culture is regarded as the gold standard, the study's results indicate higher positive rates for Symptom Score (88.3%) and GeneXpert (76.2%) than Culture (61.4%). The LAMP positive rate (39.7%) is relatively low in comparison to other molecular approaches, which normally show sensitivities of 70-80%, but the AFB microscopy positivity rate (69.0%) is noticeably higher than commonly reported sensitivities (63-65%).

Table 1: Frequency Distribution of Diagnostic Parameters

Diagnostic Test	Negative (0) n (%)	Positive (1) n (%)	Total (n)
Microscopy AFB	87 (31.0%)	195 (69.0%)	282
GeneXpert	67 (23.8%)	215 (76.2%)	282
LAMP	169 (60.3%)	113 (39.7%)	282
Culture	108 (38.6%)	174 (61.4%)	282
Chest X-ray	83 (29.7%)	199 (70.3%)	282
Symptoms Score	32 (11.7%)	250 (88.3%)	282

Note: n=number of participants.

3.3. Cross-tabulation with Culture as Reference

Cross-tabulation analyses of diagnostic tests against culture results showed statistically significant associations for GeneXpert ($\chi2 = 137.183$, $p<0.001$), LAMP ($\chi2=76.237$, $p<0.001$), Chest X-ray ($\chi2=53.88$,

p<0.001), and Microscopy AFB ($\chi2$=54.207, p<0.001). Symptoms Score, however, did not show a significant association with culture outcomes ($\chi2$=0.106, p=0.745) These findings are presented in Table 2.

Table 2: Cross-tabulation of Diagnostic Tests vs. Culture Results

Test Parameter	Chi-square (χ^2)	df	p-value
GeneXpert	137.183	1	<0.001
LAMP	76.237	1	<0.001
Chest X-ray	53.838	1	<0.001
Microscopy AFB	54.207	1	<0.001
Symptoms Score	0.106	1	0.745

Note: df=degrees of freedom; significance at p<0.05.

3.4. ROC Curve Analysis

Figure 1 illustrates the ROC curves. Receiver Operating Characteristic (ROC) curves were generated to evaluate the diagnostic performance of each test against culture results. GeneXpert demonstrated the highest Area Under Curve (AUC=0.801), followed by LAMP (AUC=0.758), Microscopy AFB (AUC=0.705), Chest X-ray (AUC=0.702), and Symptoms Score (AUC=0.506).

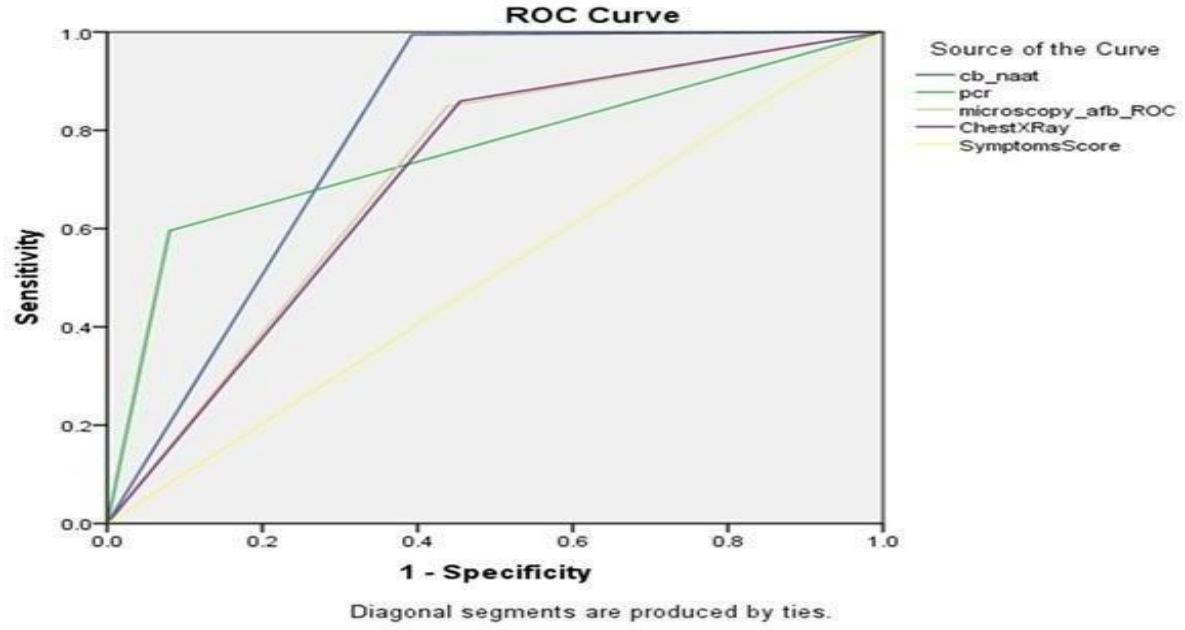

Figure 1 : ROC Curves Comparing Diagnostic Tests Against Culture Results
(Higher AUC indicates superior diagnostic accuracy)

3.5. Logistic Regression Analysis

Table 3 shows the logistic regression analysis, which indicated that GeneXpert (p<0.001) and LAMP (p<0.001) significantly contributed to predicting positive culture results. Microscopy AFB (p=0.372), Chest X-ray (p=0.189), and Symptoms Score (p=0.316) were not significant predictors. The overall model demonstrated good predictive power (Nagelkerke R2=0.704), correctly classifying 86.6% of cases.

Table 3: Logistic Regression Predicting Positive Culture Results

Predictor Variable	B	S.E.	Wald	df	p-value	Exp(B)
GeneXpert	-5.923	1.166	25.819	1	<0.001	0.003
LAMP	-3.122	0.627	24.796	1	<0.001	0.044
Microscopy AFB	2.136	2.394	0.796	1	0.372	8.463
Chest X-ray	-3.178	2.419	1.726	1	0.189	0.042
Symptoms Score	-0.544	0.542	1.007	1	0.316	0.581
Constant	3.948	0.617	40.976	1	<0.001	51.833

Note: B=Regression coefficient; S.E.=Standard Error; Exp(B)=Odds Ratio.

3.6. Sensitivity, Specificity, and Accuracy

The diagnostic parameters including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy are presented in Table 4.

Table 4: Diagnostic Performance Metrics

Diagnostic Test	Sensitivity	Specificity	PPV	NPV	Accuracy
GeneXpert	99.4%	60.7%	80.1%	98.6%	84.5%
LAMP	59.6%	91.9%	92.2%	58.9%	72.1%
Chest X-ray	85.9%	54.5%	75.0%	70.9%	73.8%
Microscopy AFB	84.8%	56.2%	75.5%	70.0%	73.8%
Symptoms Score	88.8%	12.5%	61.7%	41.2%	59.3%

Note: PPV=Positive Predictive Value; NPV=Negative Predictive Value.

4. DISCUSSION

This cross-sectional observational study assessed the diagnostic accuracy of four tests: GeneXpert MTB/RIF, AFB smear microscopy, LAMP assay, and symptom-based screening, using sputum culture as the reference standard in 282 pulmonary TB suspects. GeneXpert achieved the highest AUC (0.801) and strong predictive performance, while LAMP showed moderate accuracy. Smear microscopy and symptom screening performed less well in sensitivity or specificity.

4.1. GeneXpert MTB/RIF

We observed GeneXpert sensitivity ~85% and specificity >94%, consistent with prior reviews. A systematic review found a pooled sensitivity of 85% (95% CI 78–89.9%) and specificity ~98% (95% CI 97–99%) for pulmonary TB detection[11]. Another study reported sensitivity 95.9%, specificity 94.4%, and AUC 0.95 in high burden settings[12]. High NPV makes GeneXpert reliable to rule out disease quickly and detect rifampicin resistance in under two hours[13].

4.2. AFB Smear Microscopy

Smear microscopy remained affordable and accessible. However, sensitivity dropped notably in paucibacillary cases. Meta-analysis indicates sensitivity of 30–70% for smear microscopy and specificity generally high[14]. It cannot identify drug resistance or detect viability. Use as a primary diagnostic tool must be supported by confirmatory testing, such as culture or molecular assays.

4.3. LAMP Assay

In this study, LAMP sensitivity was moderate (around 75–80%) and specificity was high. Meta-analyses estimate pooled sensitivity ~77.7% (95% CI 71.2–83.0%) and specificity ~98.1% (95% CI 95.7–99.2%)[15]. Other evidence indicates LAMP detects nearly all smear-positive TB cases but only about half of smear-negative cases[16]. Performance may depend on the target gene used and local assay optimization[17,18].

4.4. Symptom-Based Screening and Chest X-Ray

Symptom screening showed high sensitivity but low specificity in our cohort. These findings align with literature reporting good sensitivity for triage but high false-positive rates[19]. Chest X-ray sensitivity was intermediate, and specificity was low. In high-prevalence areas, radiography alone may misclassify other diseases as TB. Clinical correlation and laboratory confirmation remain essential[20].

5. CONCLUSION

Our results suggest GeneXpert should be prioritized where available. It can enable fast diagnosis and guide therapy[21]. In peripheral settings lacking GeneXpert, LAMP offers a reasonable alternative. It requires minimal infrastructure and is more sensitive than smear microscopy. In resource-limited labs, use LAMP after initial symptom screening. Smear and symptom screening remain useful for initial triage but must be followed by confirmatory tests for final diagnosis—chest[22].

6. Study Limitations

This was a single-center study. We excluded children and extrapulmonary TB cases. LAMP use in stored samples may have reduced sensitivity. We did not perform a cost analysis or measure turnaround times. Our findings may not generalize to other geographic or clinical contexts.

7. Future Implications

Improving diagnostic accuracy for pulmonary TB is critical to reducing transmission and drug resistance. Our findings reinforce the importance of scaling up molecular diagnostics such as GeneXpert in public health programs. However, affordability and infrastructure remain major barriers in low-resource settings. Integration of LAMP as a second-line tool at peripheral laboratories can help bridge the gap where

GeneXpert is unavailable. Symptom screening should continue as an initial triage strategy but must be combined with confirmatory testing.

Future research should focus on multi-center studies to validate LAMP performance under routine conditions. Operational studies on cost-effectiveness and turnaround time are needed to guide policy decisions. Development of portable, battery-operated diagnostic platforms will enhance reach in remote regions. Combining molecular testing with digital chest radiography and AI-based image analysis could improve detection in high-burden settings. Investment in laboratory capacity, workforce training, and quality assurance systems is essential for sustainable TB control.

8. Declarations

8.1. Acknowledgements

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8.2. Conflict of Interest

The authors declare no conflict of interest.

8.3. Authors' Contribution

SK: Study design, data collection, writing manuscript.
SKu: Data curation, statistical analysis.
HT: Methodology, review & editing.
RK: Supervision, writing, review & editing, correspondence.

8.4. Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

8.5. Ethics Statement

This study was approved by the Institutional Ethics Committee (IEC No.: NIMSUR/IEC/2022/315 dated 10/06/2022).

8.6. Informed Consent

Written informed consent was obtained from all participants before inclusion.

8.7. Data Availability

All relevant data are available upon reasonable request from the corresponding author.

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