ORIGINAL RESEARCH PAPER
Volume - 14 | Issue - 11 | November - 2025 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

COMPARISON OF GENEXPERT MTB/RIF AND FIRST-LINE LINE PROBE ASSAY FOR DETECTION OF MYCOBACTERIUM TUBERCULOSIS AND RIFAMPICIN RESISTANCE FROM CLINICALLY SUSPECTED CASES OF PULMONARY AND EXTRAPULMONARY TUBERCULOSIS

Medical Microbiology

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ABSTRACT

Background: Molecular methods like GeneXpertMTB/RIF and FL-LPA, endorsed by WHO for diagnosing drug-resistant tuberculosis. AIM: Evaluation of diagnostic performance of GeneXpertMTB/RIF and FL-LPA in comparison with liquid culture, the "gold standard" for diagnosing pulmonary and extrapulmonary tuberculosis. **Methods:** In total 2912 pulmonary and 2522 extrapulmonary samples were subjected to GeneXpert MTB/RIF upfront, of which 388 were AFB smear positive, processed for direct LPA. A total 2524 pulmonary samples and 2522 extrapulmonary samples were subjected to liquid culture, of which samples flagged out as culture positive were put for FL-LPA. **Results:** Concordance was 100% in GeneXpertMTB/RIF and FL-LPA for AFB smear positive pulmonary samples. The sensitivity and specificity of GeneXpert MTB/RIF for pulmonary samples was found 91.39% and 94.49%, PPV and NPV were 82.07% & 97.55%. For extrapulmonary samples, sensitivity & specificity were 78.48% & 93.20%. PPV & NPV were 68.11% & 95.90%. The sensitivity of FL-LPA for pulmonary samples was 98.35%, PPV was 100%, for extrapulmonary samples sensitivity was 97.64%, PPV was 100%. Rifampicin resistance detection by GeneXpert MTB/RIF in 24.52% of total samples. In FL-LPA, MDR-TB, Mono-rifampicin resistance was seen in 22.62% & 1.85% cases, respectively. **Conclusion:** The study reinforces reliability on GeneXpert MTB/RIF and LPA as rapid diagnostic tools in drug resistance detection. GeneXpert with its rapid turnaround time and ease of use is especially valuable in primary and peripheral settings, while LPA offers early and detailed drug resistance.

KEYWORDS

GeneXpert MTB/RIF, FL-LPA, Pulmonary tuberculosis, Extrapulmonary tuberculosis, Rifampicin

INTRODUCTION

Tuberculosis is one of the oldest diseases known to affect humans caused by bacteria of the *Mycobacterium tuberculosis* complex[1]. About a quarter of the global population is estimated to have been infected with TB[2]. According to WHO'S 2024 global tuberculosis report, TB caused an estimated 1.25 million (95% UI: 1.13–1.37 million) deaths. Five countries accounted for 56% of the global total, India having the highest case burden.[3]

In 2021, the Government of India introduced 'Guidelines for programmatic management of drug- resistant tuberculosis in India' with an aim of aligning it to World Health Organization end TB strategy recommending the use of cartridge based nucleic acid amplification test as the first line test for screening of patients for M. tb and detecting Rif resistance, followed by line probe assay to confirm Rif resistance and to detect isoniazid (H) resistance. [4] Culture remains the reference standard for TB diagnosis.[5] However, it is a time-intensive process, requiring 2-3 months to provide results. To overcome these limitations, there has been a substantial shift towards utilizing rapid molecular diagnostics tests such as the line probe assay (GenoType MTBDR plus, Hain Lifescience, Nehren, Germany) and the GeneXpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA)[6]

The aim of the study is to compare GeneXpert MTB/RIF and FL-LPA for the detection of MTB and rifampicin resistance in clinically suspected cases of MDR-TB, with objectives to determine sensitivity, specificity, positive predictive value and negative predictive value of GeneXpert and FL-LPA using MGIT culture as the "gold standard" and to detect rifampicin resistance by GeneXpert and FL-LPA for *Mycobacterium tuberculosis*.

METHODOLOGY:

A prospective observational study was conducted in TB culture and DST laboratory of a tertiary care hospital. Institutional ethics committee approval was taken. A total of two thousand nine hundred twelve pulmonary and two thousand five hundred twenty-two extrapulmonary samples from clinically suspected cases of MDR-TB during the period from September 2022 to February 2024 were included in the study. As per the diagnostic algorithm under NTEP, upfront GeneXpert MTB/RIF of all the samples was done.

All samples were subjected to decontamination and concentration method. All ZN smear positive sputum samples were subjected to direct LPA. All smear negative sputum samples, other pulmonary samples and extrapulmonary samples were subjected to liquid culture, whose culture isolates were put for indirect LPA. Statistical analysis was done using MedCalc's software.

RESULTS:

A total of 2,912 pulmonary samples and 2,522 extrapulmonary samples were included. Among the 2,912 pulmonary samples analyzed: 1,851 (63.53%) samples were sputum samples, 669 (22.98%) samples were gastric lavage, 252 (8.65%) were bronchoalveolar lavage samples, 131 (4.5%) were tracheobronchial secretions and 9 (0.3%) samples were post-bronchoscopy sputum samples.

All 2,912 pulmonary and 2,522 extrapulmonary samples were tested using GeneXpert MTB/RIF as part of the diagnostic algorithm under the National Tuberculosis Elimination Program (NTEP).

Of 1,851 GeneXpert tested sputum samples, 388 were AFB-positive by Ziehl-Neelsen staining. When these 388 samples underwent direct first-line Line Probe Assay (LPA), results were concordant with GeneXpert perfectly for *Mycobacterium tuberculosis* detection and rifampicin resistance.

Of the 2912 pulmonary samples, except for 388 ZN smear positive sputum samples, 1463 sputum samples that tested negative for acid-fast bacilli by Ziehl-Neelsen staining, as well as gastric lavage, bronchoalveolar lavage, tracheobronchial secretions, post-bronchoscopy samples, and 2522 extrapulmonary samples, which included 569 pleural fluid, 516 CSF, 511 pus, 397 lymph node, 212 tissue, 210 ascitic fluid, 38 peritoneal fluid, 24 synovial fluid , 19 pericardial fluid, 12 bone marrow, 10 drain fluid, 4 bone were processed for liquid culture using the BD BACTEC MGIT 960 Mycobacteria Culture System (Sparks, Maryland, USA). The samples which flagged positive in liquid culture were processed for indirect FL-LPA.

Results of all the samples which showed *Mycobacterium tuberculosis* detected in GeneXpert/RIF, liquid culture and FL- LPA have been shown below in table 1.

Table 1: Total Samples Detected Positive For *Mycobacterium Tuberculosis* By GeneXpert MTB/RIF , Liquid Culture And First Line Line Probe Assay (N = 2881)

SAMPLES	GENEXPERT MTB/RIF	LIQUID CULTURE	FL-LPA
SPUTUM	515	456	447
GASTRIC LAVAGE	32	31	30
BRONCHOALVEOLAR LAVAGE	40	40	39
TRACHEOBRONCHIAL SECRETIONS	18	15	15
POST BRONCHOSCOPY SPUTUM	3	4	4
PUS	143	100	98
TISSUE	34	33	33
LYMPH NODE	117	95	93
PLEURAL FLUID	96	95	91
CSF	40	47	46
ASCITIC FLUID	7	9	9
SYNOVIAL FLUID	2	2	2
PERICARDIAL FLUID	0	0	0
BONE MARROW	0	0	0
PERITONEAL FLUID	0	0	0
DRAIN FLUID	0	0	0
BONE	0	0	0
TOTAL	1047	927	907

As seen in table 2 , Of 2524 pulmonary samples, 499 were positive for *Mycobacterium tuberculosis* in both culture and Xpert MTB/RIF, and 1,869 samples were negative by both methods. Among the 608 samples detected by Xpert MTB/RIF, 109 did not show growth of *Mycobacterium tuberculosis* in culture. Additionally, out of 1,916 samples, 47 were positive for *Mycobacterium tuberculosis* in culture but were not detected by Xpert MTB/RIF.

In Pulmonary samples, GeneXpert MTB/RIF when compared with liquid culture (MGIT), demonstrated a sensitivity of 91.39% and a specificity of 94.49%. The positive predictive value was 82.07%, while the negative predictive value was 97.55%.

Table 2 : Comparative Evaluation Of Xpert MTB/RIF With Liquid Culture (MGIT) For Pulmonary Samples (N = 2524)

XPERT MTB/RIF	Liquid Culture		Total
	Positive	Negative	
Positive	499	109	608
Negative	47	1869	1916
TOTAL	546	1978	2524

A total of 546 pulmonary samples which flagged positive in liquid culture were subjected to FL-LPA, as seen in table 3, Out of 546 pulmonary samples, 535 tested positive for *Mycobacterium tuberculosis* by both culture and FL-LPA. While 11 samples were not detected by FL-LPA , The samples that were negative in MGIT culture had no corresponding culture isolates for indirect LPA, so specificity and negative predictive values could not be calculated.

In pulmonary samples, FL-LPA, when compared with liquid culture (MGIT), demonstrated a sensitivity of 98.35% and positive predictive value of 100%.

Table 3: Comparative Evaluation Of FL-LPA With Liquid Culture (MGIT) For Pulmonary Samples

FL-LPA	Liquid Culture		TOTAL
	Positive	Negative	
Positive	535	00	535
Negative	11	00	11
TOTAL	546	00	546

Amongst 2,522 extrapulmonary samples, 299 were positive for *Mycobacterium tuberculosis* in both culture and Xpert MTB/RIF, while 2001 samples were negative by both methods. Among the 439 samples detected positive by Xpert MTB/RIF, 140 (5.55%) did not grow *Mycobacterium tuberculosis* in culture. Additionally, out of 2,083 samples not detected on GeneXpert MTB/RIF, 82 were positive for *Mycobacterium tuberculosis* in culture (table 4)

Table 4 : Comparative Evaluation Of Xpert MTB/RIF With Liquid Culture (MGIT) for All Extrapulmonary Samples (N = 2522)

XPERT MTB/RIF	Liquid Culture		TOTAL
	Positive	Negative	
Positive	299	140	439
Negative	82	2001	2083
TOTAL	381	2141	2522

For extrapulmonary samples, GeneXpert MTB/RIF, when compared with liquid culture (MGIT), showed a sensitivity of 78.48% and a specificity of 93.20%. The positive predictive value was 68.11%, while negative predictive value was 95.90%, respectively.

As seen in table 5, Out of 381 samples which showed growth in liquid culture, 372 samples were detected positive for Mycobacterium tuberculosis in both culture and FL-LPA, 9 samples could not be detected by FL-LPA. The samples that flagged negative in MGIT culture, had no culture isolates to be put for indirect LPA. Hence, specificity and Negative predictive values could not be calculated, while. Sensitivity was 98.41 % and positive predictive value was found to be 100%.

Table 5 : Comparative Evaluation Of FL-LPA With Liquid Culture (MGIT) For All Extrapulmonary Samples (N = 381)

FL-LPA	Liquid Culture		TOTAL
	Positive	Negative	
Positive	372	00	372
Negative	09	00	09
TOTAL	381	00	381

Rifampicin Resistance Detection

Rifampicin resistance was detected in 352 samples (24.52%) of the 1435 samples that were positive by GeneXpert MTB/RIF. Out of 1295 samples that tested positive for *Mycobacterium tuberculosis* by FL-LPA, 293 samples (22.63%) were detected as MDR-TB.

DISCUSSION:

The study was designed to evaluate the accuracy of molecular methods— GeneXpert MTB/RIF and FLLPA—by comparing them with the "gold standard" liquid culture method and includes a comparatively larger sample size and inclusive of pulmonary and extrapulmonary samples. Among the 1851 sputum samples analysed in the present study, 388 were identified as AFB-positive, all of which demonstrated 100% concordance between GeneXpert MTB/RIF and first-line Line Probe Assay (FL-LPA) results. In contrast, Aricha et al. reported a lower concordance rate of 85% between the two diagnostic modalities, highlighting the superior agreement observed in our study.[3] In Aricha et Al's study, GeneXpert showed a sensitivity of 78.5%, specificity of 99.6%, a positive predictive value of 81.3%, and a negative predictive value of 98.9%. Our study shows comparable specificity, negative predictive value with marginal differences and higher sensitivity, PPV comparatively. In Yadav et al.'s study, GeneXpert showed a sensitivity of 76.5% (50.1% – 93.2 %), specificity of 94.6 % (89.6 % - 97.6%), Positive predictive value of 97.9% (96.0- 98.9 %) and negative predictive value of 95.2 % (90.6% – 97.7%).[3,7] Our study shows higher sensitivity and NPV, comparable specificity and lower PPV as compared to Yadav et al's study.

In the study by Aricha et al, the LPA test in their study exhibited a sensitivity of 99.2% and a specificity of 26.9%, with a positive predictive value of 70.7% and a negative predictive value of 94.7%. Our study is in concordance with Aricha et Al's study showing a higher sensitivity for FL-LPA for pulmonary samples. In a study conducted by Yadav et al. LPA had a sensitivity of 66.7%, specificity of 96.0 %, positive predictive value of 98.4 % and negative predictive value of 93.5 %. Our study is in disconcordance with the study of Yadav et Al's which shows lower sensitivity. [3,7] Our study shows a higher PPV in comparison to both studies.

In a study conducted by Sekyere et al. that GeneXpert had a sensitivity of 53.85% and specificity of 98.51%, while the positive predictive value was 69.23%, and the negative predictive value was 50.00%.[8] Our study has significantly higher sensitivity and NPV, and comparable specificity and PPV with marginal difference than their values. Mafijuddin et al observed sensitivity of 83.7%, specificity of 96.0 %, positive predictive value of 71.1 % and negative predictive value of 98 % for GeneXpert MTB/RIF for extrapulmonary samples.[9] Our study has lower sensitivity and PPV, and comparable specificity and NPV. In similar study conducted by Tortoli et al, sensitivity and specificity of

GeneXpert MTB/RIF for extrapulmonary samples was found to be 81.3 % and 99.8 % respectively.[10] Our study has lower sensitivity and higher specificity in comparison.

For FLLPA, the study conducted by Sekyere et al. demonstrated sensitivity and specificity as 69.23% and 49.25%, with a negative predictive value of 97.01% and a positive predictive value of 25.37%. Our study shows a higher sensitivity for LPA, which could be due to the use of indirect LPA, which tests culture isolates, unlike the direct LPA method used in Sekyere et al.'s study, where sample concentrates were used[8].

Our study observed that GeneXpert MTB/RIF identified rifampicin resistance in 29.15% of extrapulmonary samples and 22.48% of pulmonary samples, with rifampicin resistance of 24.52 % for all samples. This is in concordance to a study by Kanade et al., who found resistance rates of 24.71% in extrapulmonary and 25.18% in pulmonary samples.[11] The higher resistance rate in extrapulmonary samples in our study could be linked to the challenges of managing multidrug-resistant (MDR) tuberculosis and the lack of transmission control measures. The use of GeneXpert under the National Tuberculosis Elimination Program (NTEP) has contributed to increased detection of rifampicin resistance, enhancing clinical management.[12] In earlier studies conducted by Kaur et al, reported a rifampicin resistance rate of 9.9% in Punjab, while Chakraborty et al, noted 9.09% resistance in extrapulmonary and 13.7% in pulmonary samples which is low compared to our study (24.52%), hence displaying the increasing rifampicin resistance over a few years and emphasizing importance of rapid diagnosis of rifampicin resistance.[13,14] Overall, GeneXpert MTB/RIF is recognized for its capability to detect rifampicin resistance more quickly and accurately compared to other diagnostic methods. In our study, multidrug-resistant tuberculosis (MDR-TB) was detected in 22.62% of cases, mono isoniazid resistance in 4.56%, and mono rifampicin resistance in 1.85%. In comparison, Bellad et al. identified MDR-TB in 19.29% of cases which is lower than our detection rate and mono rifampicin resistance in 7.14%, and mono isoniazid resistance in 19.29% using first-line Line Probe Assay which is higher than our study.[15] Jain et al. reported a higher prevalence, with 34.5% of cases being MDR-TB and 40.3% exhibiting rifampicin resistance by FL-LPA .[16] Kumar et al. found 24.1% MDR-TB which is consistent with the findings of our study and 33.3% mono isoniazid resistance, and 43.1% mono rifampicin resistance values appreciably greater than that documented in our study.[17] Rufai et al. observed MDR-TB in 25.8% of cases which parallels with our observations, and 10.4% with mono isoniazid resistance, and 22.2% with mono rifampicin resistance which are higher relative to our findings. Swarnakar et al. detected mono isoniazid resistance in 32% of their study group using FL- LPA which is again a higher magnitude than that recorded in our study.[18]

CONCLUSION:

GeneXpert MTB/RIF being rapid, requiring less training, and having lower biosafety requirements than liquid culture, is ideal for initial tuberculosis testing, even in remote areas. For smear-positive sputum samples, where bacillary load is high, direct LPA is valuable for rapidly diagnosing MDR-TB. In cases of extrapulmonary samples, correlating GeneXpert results with liquid culture results is crucial. FL-LPA detects additional isoniazid resistance along with rifampicin resistance, hence detecting MDR-TB, RR-TB as well as Hr-TB , Indirect LPA from culture isolates shows higher sensitivity when compared to liquid culture and faster turnaround time than DST. In high tuberculosis burden countries, molecular methods add greater value as they contribute to rapid diagnosis and treatment initiation. Considering their strong performance in comparison to the “gold standard”, both methods contribute improved patient outcomes and more effective TB control strategies.

Acknowledgement

We confirm that all authors meet the authorship criteria. We are thoroughly thankful to the technical staff of TB Culture and DST laboratory for their thorough cooperation

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