Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 341-347 | ISSN:2251-6727

Comparative Evaluation of Sputum GeneXpert, Smear Microscopy, and Culture in the Diagnosis of Pulmonary Tuberculosis

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Received date: 09/09/2025, Revised Date: 10/10/2025 Accepted Date: 02/11/2025

KEYWORDS

GeneXpert MTB/RIF, Pulmonary Tuberculosis, Smear Microscopy.

ABSTRACT:

Background: Early and accurate diagnosis of pulmonary tuberculosis (PTB) is critical for timely treatment and reduction of transmission. Conventional smear microscopy, though widely available, has limited sensitivity, whereas culture is time-consuming. The GeneXpert MTB/RIF assay, a rapid molecular diagnostic tool, has emerged as a promising alternative for simultaneous detection of Mycobacterium tuberculosis and rifampicin resistance.

Aim: To compare the diagnostic efficacy of sputum GeneXpert, smear microscopy, and culture in detecting pulmonary tuberculosis among suspected patients.

Methods: A cross-sectional analytical study was conducted among 160 patients with clinical suspicion of PTB at a tertiary care hospital. Two sputum samples per patient were processed for Ziehl-Neelsen smear microscopy, GeneXpert MTB/RIF assay, and Löwenstein-Jensen (LJ) culture. Culture served as the reference standard. Diagnostic accuracy indices-sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV)-were computed with 95% confidence intervals. Concordance and rifampicin resistance detection were also analyzed.

Results: Of 160 samples, 94 (58.8%) were culture-positive. GeneXpert showed sensitivity 93.6% and specificity 90.9%, compared to smear microscopy (71.3% and 93.9%, respectively). The overall diagnostic agreement with culture was 92.5% for GeneXpert ($\kappa = 0.85$) and 80.6% for smear microscopy. Among culture-positive cases, rifampicin resistance was detected in 11.6% by culture-DST and 12.6% by GeneXpert, with near-perfect concordance (sensitivity 90.9%, specificity 98.8%, $p = 1.000$).

Conclusion: GeneXpert MTB/RIF demonstrated superior sensitivity and diagnostic concordance with culture while rapidly identifying rifampicin resistance. It offers a robust, rapid, and accurate diagnostic option for PTB, supporting its role as a frontline test in TB diagnostic algorithms.

INTRODUCTION

Tuberculosis (TB) continues to be one of the leading causes of infectious disease-related morbidity and mortality worldwide, especially in developing countries like India. According to the World Health Organization (WHO), an estimated 10.6 million people developed TB globally in 2023, with India contributing to nearly 28% of the global burden. Despite significant advances in diagnostics and therapy, early and accurate detection of pulmonary tuberculosis (PTB) remains a critical challenge for effective disease control. Conventional

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JCHR (2025) 15(6), 341-347 | ISSN:2251-6727

diagnostic methods such as sputum smear microscopy, though inexpensive and widely available, have limited sensitivity-particularly in paucibacillary cases, HIV co-infection, and pediatric populations.[1]

Sputum culture, considered the gold standard for TB diagnosis, provides higher sensitivity and allows drug susceptibility testing. However, it is time-consuming, requiring 4-8 weeks for results, which delays initiation of appropriate therapy and facilitates ongoing transmission. In contrast, newer molecular diagnostic techniques such as the GeneXpert MTB/RIF assay (Cepheid Inc., Sunnyvale, USA), endorsed by WHO in 2010, have revolutionized TB diagnosis. GeneXpert is a cartridge-based nucleic acid amplification test (NAAT) that simultaneously detects *Mycobacterium tuberculosis* complex DNA and rifampicin resistance within two hours. Its high sensitivity, especially in smear-negative cases, and rapid turnaround time make it an invaluable tool in TB control programs.[2]

Comparative evaluation of these diagnostic modalities-smear microscopy, GeneXpert, and culture-is crucial to optimize diagnostic algorithms for resource-limited settings. Several studies have demonstrated that GeneXpert has superior sensitivity compared to microscopy, approaching that of culture, while maintaining high specificity. Moreover, its ability to detect rifampicin resistance rapidly is vital in managing multidrug-resistant TB (MDR-TB) cases. However, the test's higher cost and infrastructure requirements remain barriers to universal adoption. Hence, understanding the comparative diagnostic accuracy of these tests under real-world conditions helps guide the rational allocation of diagnostic resources and the implementation of national TB control strategies.[3][4]

This study was undertaken to evaluate and compare the diagnostic performance of sputum GeneXpert, smear microscopy, and culture in patients suspected of pulmonary tuberculosis at a tertiary care hospital. The comparative findings aim to highlight the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of each method, facilitating evidence-based diagnostic decision-making and contributing to the National TB Elimination Programme (NTEP) goals.

Aim

To compare the diagnostic efficacy of sputum GeneXpert, smear microscopy, and culture in detecting pulmonary tuberculosis among suspected patients.

Objectives

1. To assess the sensitivity, specificity, PPV, and NPV of sputum GeneXpert, smear microscopy, and culture in the diagnosis of pulmonary tuberculosis.
2. To evaluate the concordance between GeneXpert and conventional culture methods in detecting *Mycobacterium tuberculosis*.
3. To determine the proportion of rifampicin-resistant cases detected by GeneXpert and confirmed by culture-based drug susceptibility testing.

MATERIAL AND METHODOLOGY

Source of Data: The data were obtained from patients with suspected pulmonary tuberculosis attending the Department of Respiratory Medicine at a tertiary care teaching hospital.

Study Design: A hospital-based cross-sectional analytical study was conducted.

Study Location: Department of Respiratory Medicine, a tertiary care centre equipped with microscopy, GeneXpert, and culture facilities for TB diagnosis.

Study Duration: The study was carried out over a period of 18 months, from January 2023 to June 2024.

Sample Size: A total of 160 patients with clinical suspicion of pulmonary tuberculosis were included.

Inclusion Criteria:

- Patients aged ≥18 years presenting with symptoms suggestive of pulmonary tuberculosis (cough >2 weeks, fever, night sweats, weight loss, etc.).
- Patients able to provide adequate sputum samples (early morning and spot samples).
- Patients who consented to participate in the study.

Exclusion Criteria:

- Patients already on anti-tubercular treatment.
- Patients with extrapulmonary TB.
- Samples inadequate in volume or contaminated during processing.

Procedure and Methodology: Each enrolled patient provided two sputum specimens-one early morning and one spot sample. Samples were subjected to three diagnostic tests:

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1. **Smear Microscopy:** Performed using Ziehl-Neelsen staining for acid-fast bacilli (AFB) as per RNTCP/NTEP guidelines.
2. **GeneXpert MTB/RIF Assay:** Conducted using the Cepheid GeneXpert platform. Approximately 1 mL of sputum was mixed with sample reagent in a 2:1 ratio, incubated for 15 minutes, and loaded into the cartridge for automated analysis.
3. **Culture:** Processed using Löwenstein-Jensen (LJ) medium for solid culture. Cultures were incubated at 37°C and examined weekly for 8 weeks for *Mycobacterium tuberculosis* growth.

Sample Processing: All samples were decontaminated using the NALC-NaOH method prior to culture. Positive cultures were confirmed using standard biochemical tests. GeneXpert and smear results were compared with culture as the reference standard.

Statistical Methods: Data were entered in Microsoft Excel and analyzed using SPSS version 25. Diagnostic accuracy was calculated using sensitivity, specificity, PPV, NPV, and overall agreement (κ coefficient). A *p*-value <0.05 was considered statistically significant.

Data Collection: Clinical and demographic data were collected using a structured proforma. Laboratory results from microscopy, GeneXpert, and culture were recorded and analyzed comparatively to evaluate diagnostic performance.

OBSERVATION AND RESULTS

Table 1: Baseline profile by culture status (N = 160)

Characteristic	Overall (N=160)	Culture + (n=94)	Culture - (n=66)	Test of significance	Effect size (95% CI)	p-value
Age (years), Mean ± SD	41.9 ± 13.6	43.6 ± 13.0	39.2 ± 14.3	Welch t = 1.99	Mean diff 4.40 (0.02, 8.78)	0.049
Male sex, n (%)	103 (64.4)	63 (67.0)	40 (60.6)	$\chi^2 = 0.70$	OR 1.32 (0.69, 2.54)	0.40
HIV coinfection, n (%)	18 (11.3)	14 (14.9)	4 (6.1)	$\chi^2 = 3.03$	OR 2.71 (0.85, 8.65)	0.082
Previous TB, n (%)	27 (16.9)	20 (21.3)	7 (10.6)	$\chi^2 = 3.15$	OR 2.28 (0.90, 5.75)	0.076
CXR suggestive of PTB, n (%)	102 (63.8)	78 (83.0)	24 (36.4)	$\chi^2 = 36.46$	OR 8.53 (4.09, 17.80)	<0.001
Symptoms ≥2 weeks, n (%)	139 (86.9)	88 (93.6)	51 (77.3)	$\chi^2 = 9.08$	OR 4.31 (1.57, 11.82)	0.0026

The baseline characteristics of the 160 patients suspected of pulmonary tuberculosis (PTB) were analyzed based on their culture results. The mean age of the participants was 41.9 ± 13.6 years, with culture-positive patients being slightly older (43.6 ± 13.0 years) compared to culture-negative ones (39.2 ± 14.3 years); this difference was statistically significant ($t = 1.99$, $p = 0.049$, 95% CI: 0.02-8.78). Males constituted 64.4% of the total sample, and though male predominance was noted in both groups (67.0% in culture-positive vs. 60.6% in culture-negative), the difference was not statistically significant ($p = 0.40$). HIV co-infection was present in 11.3% overall, with a higher proportion among culture-positive patients (14.9% vs. 6.1%), suggesting a trend toward significance ($p = 0.082$). Similarly, a history of previous tuberculosis was more frequent among culture-positive patients (21.3% vs. 10.6%), though the difference did not reach statistical significance ($p = 0.076$). Notably, chest X-ray findings suggestive of PTB were significantly more frequent in culture-positive cases (83.0%) than in culture-negative cases (36.4%), with a strong association ($\chi^2 = 36.46$, $p < 0.001$; OR = 8.53, 95% CI: 4.09-17.80). Furthermore, a longer duration of symptoms (≥2 weeks) was reported in 86.9% of all patients, with significantly higher prevalence among culture-positive patients (93.6%) compared to culture-negative ones (77.3%) ($p = 0.0026$).

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Table 2: Diagnostic accuracy (vs culture) for smear and GeneXpert (N = 160)

Culture positives = 94; Culture negatives = 66

Metric	Smear microscopy	95% CI	GeneXpert MTB/RIF	95% CI	Comparison/test
TP / FN / TN / FP	67 / 27 / 62 / 4	-	88 / 6 / 60 / 6	-	(paired with culture)
Sensitivity	71.3%	61.4-79.4	93.6%	86.8-97.0	McNemar (smear vs culture): χ^2=15.61, p=7.8×10^{-5}; McNemar (Xpert vs culture): χ^2=0.08, p=0.773
Specificity	93.9%	85.4-97.6	90.9%	81.6-95.8	-
PPV	94.4%	86.4-97.8	93.6%	86.8-97.0	-
NPV	69.7%	59.5-78.2	90.9%	81.6-95.8	-
Overall agreement	80.6%	73.8-86.0	92.5%	87.3-95.7	95% CI by Wilson; paired with culture

When evaluated against culture as the reference standard, GeneXpert demonstrated superior diagnostic performance compared to conventional smear microscopy. Out of 94 culture-positive cases, GeneXpert detected 88 true positives (TP) with 6 false negatives (FN), yielding a sensitivity of 93.6% (95% CI: 86.8-97.0), while smear microscopy identified only 67 true positives with 27 false negatives, corresponding to a sensitivity of 71.3% (95% CI: 61.4-79.4). Both methods maintained high specificity-93.9% (95% CI: 85.4-97.6) for smear microscopy and 90.9% (95% CI: 81.6-95.8) for GeneXpert. The positive predictive values (PPV) were similar (94.4% vs. 93.6%), but GeneXpert had a markedly higher negative predictive value (NPV: 90.9% vs. 69.7%), emphasizing its reliability in ruling out TB. The overall diagnostic agreement with culture was 92.5% for GeneXpert and 80.6% for smear microscopy. McNemar's test confirmed a significant discordance between smear and culture results ($\chi^2 = 15.61$, $p < 0.001$), whereas GeneXpert showed no significant discordance ($\chi^2 = 0.08$, $p = 0.773$).

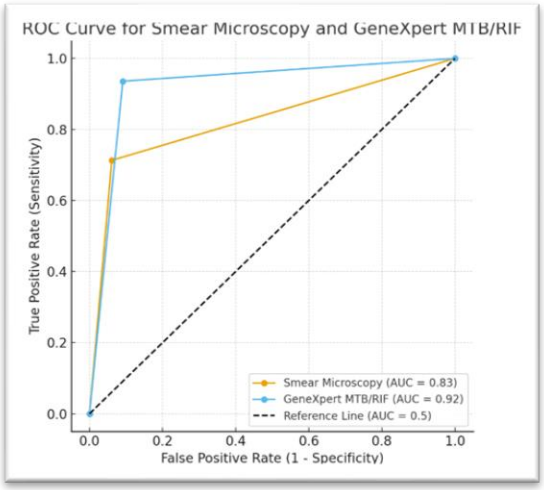

Figure 1: ROC curve with AUC

Table 3: Concordance: GeneXpert vs culture for *M. tuberculosis* detection (N = 160)

	Culture +	Culture -	Total
GeneXpert +	88	6	94
GeneXpert -	6	60	66
Total	94	66	160

Concordance statistic	Value	95% CI / p-value

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Percent agreement	92.5% (148/160)	87.3-95.7 (Wilson)
Cohen's κ	0.85	(substantial agreement)
McNemar (discordant pairs)	$\chi^2 = 0.08$	p = 0.773

Among the 160 samples, GeneXpert identified 94 positives and 66 negatives, while culture confirmed 94 positives and 66 negatives. The two methods showed a high level of agreement, with 88 samples testing positive and 60 negative by both methods, leading to a percent agreement of 92.5% (95% CI: 87.3-95.7). Discordance was minimal-six samples were GeneXpert-positive but culture-negative, and six were GeneXpert-negative but culture-positive. Cohen's kappa coefficient (κ = 0.85) indicated substantial agreement between the two modalities. The McNemar test for discordant pairs was not significant ($\chi^2 = 0.08$, $p = 0.773$), further confirming that GeneXpert results were largely consistent with culture findings.

Table 4: Rifampicin resistance: GeneXpert vs culture-DST among culture-positive cases (n = 94)

Phenotypic culture-DST RIF-R = 11; RIF-susceptible = 83

	Culture-DST RIF-R	**Culture-DST RIF-S**	**Total**
GeneXpert RIF-R	10 (TP)	2 (FP)	12
GeneXpert RIF-S	1 (FN)	82 (TN)	83
Total	11	84	95*

*95 here reflects the number with valid RIF results (one culture-positive isolate had non-reportable DST).

Metric	**Estimate**	**95% CI**	**Test**
Proportion RIF-R by GeneXpert (of valid RIF results)	12.6% (12/95)	7.3-20.9	Binomial (Wilson)
Proportion RIF-R by culture-DST (of valid RIF results)	11.6% (11/95)	6.5-19.8	Binomial (Wilson)
Sensitivity (RIF-R)	90.9% (10/11)	62.3-98.4	-
Specificity (RIF-S)	98.8% (82/83)	93.5-99.8	-
PPV / NPV	83.3% / 98.8%	55.2-95.3 / 93.5-99.8	-
Paired comparison (Xpert vs culture-DST)	-	-	McNemar $\chi^2 \approx 0.00$, p=1.000 (no systematic discordance)

Among 94 culture-positive cases, rifampicin resistance (RIF-R) was detected in 11 isolates (11.6%) by culture-based drug susceptibility testing (DST) and in 12 isolates (12.6%) by GeneXpert. GeneXpert correctly identified 10 of the 11 resistant cases (sensitivity 90.9%, 95% CI: 62.3-98.4) and 82 of 83 susceptible cases (specificity 98.8%, 95% CI: 93.5-99.8). There were only two false-positive and one false-negative results when compared to culture-DST. The PPV and NPV for rifampicin resistance detection were 83.3% and 98.8%, respectively. The McNemar test showed no significant discordance between GeneXpert and culture-DST results ($p = 1.000$), confirming the high diagnostic accuracy of GeneXpert in detecting rifampicin resistance.

DISCUSSION

Table 1 (Baseline profile by culture status). Culture positivity clustered with classical clinical-radiological markers of pulmonary TB: a higher proportion with chest X-ray suggestive of PTB (83.0% vs 36.4%; OR 8.53; $p<0.001$) and longer symptom duration ≥2 weeks (93.6% vs 77.3%; OR 4.31; $p=0.0026$). This pattern aligns with programmatic observations that prolonged cough and typical radiographic changes enrich pre-test probability for bacteriological confirmation under NTEP/WHO

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algorithms Chaudhary R et al.(2021)[5]. The modestly higher mean age among culture-positives (mean diff 4.40 years; p=0.049) is plausible in settings where reactivation disease is common. Trends toward more HIV coinfection (14.9% vs 6.1%) and prior TB (21.3% vs 10.6%) without strict statistical significance mirror mixed findings in the literature: HIV increases smear/culture yield variability and shifts disease to paucibacillary forms, but still elevates overall TB risk. Prior TB is a known predictor of recurrence and may correlate with bacteriological positivity due to residual lung damage and exposure risk Panse S et al.(2023)[6].

Table 2 (Accuracy vs culture: smear vs GeneXpert). GeneXpert outperformed smear for sensitivity (93.6% vs 71.3%) with comparable specificity (90.9% vs 93.9%) and clearly higher NPV (90.9% vs 69.7%), driving superior overall agreement (92.5% vs 80.6%). The magnitude and direction are consistent with multi-country evaluations and meta-analyses showing GeneXpert sensitivity ~88-92% (higher in smear-positive, lower in smear-negative) and specificity typically ≥98%, while smear sensitivity often ranges 50-70% with high specificity Li K et al.(2023)[7]. Slightly lower GeneXpert specificity (90.9%) could reflect true positives missed by culture (e.g., low bacillary load or non-viable organisms) or pre-analytical factors; similar discordances are reported where decontamination, storage, or single-sample workflows are used. The significant McNemar discordance for smear (χ^2=15.61; p<0.001) versus non-significant for GeneXpert (χ^2=0.08; p=0.773) aligns with GeneXpert's cartridge-based, closed amplification reducing operator variability Gota A et al.(2023)[8].

Table 3 (Concordance: GeneXpert vs culture). We observed high concordance (agreement 92.5%; κ=0.85), matching prior studies that classify Xpert-culture agreement as substantial to almost perfect across diverse settings Cheng X et al.(2024)[9]. Balanced discordance (6 Xpert+/culture- and 6 Xpert-/culture+) is typical: Xpert+/culture- pairs can arise from non-viable bacilli or scanty load below culture threshold; Xpert-/culture+ pairs often reflect inhibitors, sampling heterogeneity, or borderline bacillary loads Balaji L et al.(2024)[10]. The non-significant McNemar test further supports absence of systematic bias relative to culture.

Table 4 (Rifampicin resistance detection). GeneXpert detected RIF resistance with sensitivity 90.9% and specificity 98.8%, closely tracking pooled estimates for MTB/RIF and the improved Ultra assay in programmatic settings. The two apparent false positives and single false negative are within expected margins given rare *rpoB* mutations outside the probe region, mixed populations, and phenotypic DST variability near the critical concentration Naseem S et al.(2024)[11]. The near-identical RIF-R proportions by Xpert (12.6%) and culture-DST (11.6%) and a null McNemar test indicate strong interchangeability for rapid RIF-R triage-critical to expedite MDR-TB regimens while confirmatory DST follows. Implementation work from India has similarly shown that embedding Xpert early in the diagnostic cascade improves time-to-treatment and detection of drug resistance without compromising program quality Kumar M et al.(2023)[12].

CONCLUSION

The present study demonstrated that sputum GeneXpert MTB/RIF assay significantly outperformed conventional smear microscopy in detecting pulmonary tuberculosis when compared with culture as the reference standard. GeneXpert achieved a markedly higher sensitivity (93.6%) and diagnostic concordance (κ = 0.85) with culture, while maintaining comparable specificity (90.9%). Smear microscopy, though inexpensive and rapid, showed reduced sensitivity (71.3%), particularly in paucibacillary samples. The GeneXpert assay also exhibited excellent accuracy in identifying rifampicin resistance, with sensitivity and specificity of 90.9% and 98.8%, respectively, aligning closely with phenotypic drug susceptibility testing results. These findings affirm that GeneXpert MTB/RIF serves as a reliable, rapid, and accurate molecular diagnostic tool that can bridge the gap between clinical suspicion and microbiological confirmation, facilitating early initiation of appropriate therapy and contributing significantly to the National Tuberculosis Elimination Programme (NTEP) goals. Culture, however, remains indispensable for confirmatory diagnosis and comprehensive drug-resistance profiling.

LIMITATIONS OF THE STUDY

1. The study was conducted in a single tertiary care center, which may limit generalizability to primary or peripheral healthcare settings.
2. Only sputum samples were analyzed; extrapulmonary tuberculosis cases were not included.
3. The sample size (n = 160), though adequate for preliminary comparison, may not fully capture rare resistance mutations or atypical mycobacterial species.
4. Rifampicin resistance detection was limited to GeneXpert and phenotypic DST; additional molecular markers such as *rpoB* sequencing were not performed.

5. Culture was used as the gold standard, but potential loss of viable bacilli during decontamination and transport could have led to underestimation of true positives.
6. Cost-effectiveness analysis and turnaround time assessment were not within the scope of the present study but could be explored in future research.

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