



Original Article

Diagnostic Accuracy of Xpert Mycobacterium Tuberculosis/Rifampicin in Detecting Pulmonary Tuberculosis from Bronchoalveolar Lavage Fluid in Sputum-Negative Patients

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ABSTRACT

Pulmonary Tuberculosis (TB) is also a substantial health issue in high-burden countries like Pakistan. Diagnostic methods such as sputum smear microscopy and culture have limitations in smear-negative cases, necessitating the use of alternative methods. The Xpert MTB/RIF assay is a fast molecular diagnostic option, but its accuracy on bronchoalveolar lavage fluid (BALF) in sputum-negative patients is not well established. **Objectives:** To determine the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the Xpert MTB/RIF assay on BALF, using culture as the gold standard. **Methods:** This prospective diagnostic validation study was conducted at Lady Reading Hospital, Peshawar, from May 2025 to September 2025, including 679 adult patients with smear-negative pulmonary TB suspicion undergoing bronchoscopy. BALF samples were tested using smear microscopy, culture (gold standard), and the Xpert MTB/RIF assay. Data were analyzed using SPSS version 25.0. **Results:** Most of the patients were male 389 (57.3%). The mean age was 41.3 ± 14.2 years. Among 171 culture-positive cases, 145 were also positive by Xpert. Among 508 culture-negative cases, 490 tested negatives with Xpert. Xpert test showed 84.8% sensitivity, 96.5% specificity, 88.9% PPV, 94.9% NPV, and 93.3% diagnostic accuracy. Stratified analysis showed comparable diagnostic metrics across gender and age groups. The AUC of the ROC curve is 0.91, which indicates that Xpert performed excellently in diagnosing TB. **Conclusions:** Xpert MTB/RIF on BALF provides high diagnostic accuracy for detecting pulmonary TB in sputum-negative patients, with excellent sensitivity, specificity, and predictive values.

INTRODUCTION

Pulmonary tuberculosis (TB), caused by Mycobacterium tuberculosis (MTB), generally affects the lungs and can affect other parts of the respiratory system. According to the WHO, it remains a huge worldwide health concern, accounting for approximately 9.96 million new cases [1]. Globally, the burden by region, demographic characteristics, and underlying health conditions affects almost 25% of the population; more than 8 million people develop active TB each year [2]. About 45% of TB cases globally come from the Asia-Pacific region, with India and

Indonesia being among the countries that report the highest burden. The majority of adult men are affected, especially those above 40 years of age, more than women [3]. TB is endemic in Pakistan with an estimated 525,000 new cases annually. According to WHO, Pakistan has the fifth largest TB burden in the world, and fourth among the developing countries in terms of drug-resistant TB [4]. According to a recent study of 2020 - 2023, the prevalence of TB was 25.42% among patients of respiratory symptoms, with annual rates of 23.7%, 24.8%, 28.1%, and

25.1%, respectively [5]. Effective TB control is very important, and early and correct diagnosis is essential. Although 84% of TB cases involve the lungs, bacteriological confirmation is achieved in only 57% of global pulmonary TB (PTB) cases and about 52% in Pakistan [6]. Diagnostic methods such as smear microscopy and culture have some limitations. Smear microscopy is very rapid and specific (93.94%) but not sensitive (81.8%) in smear-negative and/or paucibacillary disease, especially in tropical settings [7]. Culture, being the gold standard of diagnostic tests, is more sensitive but takes longer and is not so widely available, often needing between 2 and 6 weeks for turnaround times for result interpretation [8]. The Xpert MTB/RIF test, approved by WHO in 2010, is a quick molecular test that can detect TB and rifampicin-resistance in two hours [9, 10]. Although very effective, its diagnostic sensitivity in smear-negative patients, for example, is not very high, at 67% in a study [11].

For patients who are unable to produce sputum or have smear-negative results, bronchoscopy provides access to lower respiratory tract secretions through BALF. This study aimed to evaluate its diagnostic accuracy in sputum-negative suspected pulmonary TB cases to help bridge a key diagnostic gap and enhance patient outcomes by giving the limited evidence on Xpert MTB/RIF performance using BALF.

METHODS

This prospective diagnostic validation study was undertaken at the Department of Pulmonology, Lady Reading Hospital, Peshawar, from May 2025 to September 2025. A total of 679 patients were recruited through consecutive sampling technique, calculated based on previously reported sensitivity and specificity of smear microscopy for pulmonary TB (81.8% and 93.94%, respectively) [5, 7], a national prevalence of 25.42% [5], a confidence level of 95%, and a desired precision of 0.06 using the WHO sample size calculator. The patient was enrolled consecutively in the study based on predefined inclusion and exclusion criteria. This approach was used to reduce selection bias and reflect routine clinical practice. The inclusion criteria were patients of either gender, age ≥ 18 years, with two consecutive sputum smear results negative for acid-fast bacilli and a clinical suspicion of pulmonary TB, and who were scheduled to undergo bronchoscopy for diagnostic purposes. Patients were excluded if they had a history of TB treatment, were known cases of extrapulmonary TB, or had contraindications to bronchoscopy. After obtaining ethical approval from the IRB committee under Ref No: 527/LRH/MTI, eligible patients were enrolled after informed consent, with assurance of confidentiality and the right to withdraw at any stage. Baseline information like age, gender, height,

weight, BMI, education, occupation, socioeconomic status, urban or rural residence, history of TB, comorbidities, presenting symptoms, and body temperature were documented. Radiological assessment with chest X-ray or CT scan was done for each patient. Initial diagnostic evaluation included two sputum smear tests for AFB; those testing negative proceeded to bronchoscopy. BALF was collected under sterile conditions and tested using the Xpert MTB/RIF assay to spot MTB DNA and rifampicin resistance. The same samples were analyzed using AFB smear microscopy and MGIT liquid culture (Becton Dickinson) as the reference/gold standard. Culture results indicating contamination, growth of samples that were unclear, or a lack of viability were censored from the analysis. BALF samples were processed immediately following sample collection. Processing of samples involved digestion-decontamination using the N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) method followed by centrifugation at $3000 \times g$ for 15 minutes. This resulted in the use of the pellet for smear microscopy analysis, Xpert MTB/RIF analysis, or MGIT culture. Alternatively, if processing in the reference lab was not done immediately, the samples were stored at $2-8^{\circ}\text{C}$ for a period of a maximum of 24 hours to prevent a decrease in viability and the development of contamination. The trained personnel performed all the laboratory tests using standardized protocols. Xpert MTB/RIF diagnostic performance data were checked against findings of a culture, and rifampicin resistance was reported for clinical management. Data were analysed through SPSS version 25.0. Categorical data were expressed as frequencies and percentages, while continuous variables were summarized as mean \pm SD and median (IQR). Diagnostic accuracy measures were derived from a 2×2 contingency table. Subgroup comparisons for diagnostic accuracy across gender and age categories were evaluated using Pearson's Chi-square test. P-values were not adjusted for multiple comparisons, given the limited number of pre-specified, independent subgroup analyses ($n=2$). Receiver operating characteristic (ROC) analysis was performed to assess the diagnostic performance of XPERT MTB/RIF using culture as the reference standard. As XPERT MTB/RIF provides a dichotomous result (positive/negative), the ROC plot represents a single operating point. The area under the curve (AUC) was estimated to quantify overall diagnostic discrimination.

RESULTS

A total of 679 adult patients were included, having a mean age of 41.3 ± 14.2 years, body temperature was $37.8 \pm 0.9^\circ\text{C}$, and BMI was $24.5 \pm 4.1 \text{ kg/m}^2$ (Table 1).

Table 1: Summary Statistics of Continuous Variables (Adults, n=679)

Variables	Mean \pm SD
Age (Years)	41.3 ± 14.2
Body Temperature ($^\circ\text{C}$)	37.8 ± 0.9
Height (cm)	166.5 ± 9.2
Weight (kg)	67.8 ± 13.4
Body Mass Index (BMI)	24.5 ± 4.1

The majority of the study participants were male 389 (57.3%) and were urban residents 410 (60.4%). Regarding socioeconomic status, most participants belonged to the middle socioeconomic group, 317 (46.7%), followed by the low group, 278 (40.9%), and a smaller proportion in the high group, 84 (12.4%). Clinically, 592 (87.2%) individuals were symptomatic, whereas 87 (12.8%) were asymptomatic at presentation. A history of tuberculosis was present in 139 (20.5%) participants. Additionally, 176 (25.9%) had comorbid conditions. Radiological evaluation showed positive findings in 428 (63.0%) participants, while 251 (37.0%) had negative radiological results (Table 2).

Table 2: Baseline Characteristics (n=679)

Variables	Categories	(n) %
Gender	Male	389 (57.3%)
	Female	290 (42.7%)
Residency	Urban	410 (60.4%)
	Rural	269 (39.6%)
Socioeconomic Status	Low	278 (40.9%)
	Middle	317 (46.7%)
	High	84 (12.4%)
Presenting Status	Symptomatic	592 (87.2%)
	Asymptomatic	87 (12.8%)
History of TB	–	139 (20.5%)
Comorbid Conditions	–	176 (25.9%)
Radiological Findings	Positive	428 (63.0%)
	Negative	251 (37.0%)

Out of 171 patients who were positive according to the culture, 145 also tested positive with Xpert (True Positives), while 26 were missed by the test (False Negatives). Among 508 culture-negative patients, 490 were correctly identified as negative by Xpert (True Negatives), and 18 were incorrectly identified as positive (False Positives). An exploratory analysis was conducted to identify possible clinical and radiological factors contributing to discordant Xpert MTB/RIF results. Among the 18 false-positive cases, a substantial proportion had radiological abnormalities suggestive of prior pulmonary tuberculosis and/or a

documented history of previous TB treatment, raising the possibility of detection of residual mycobacterial DNA rather than active disease. Among the 26 false-negative cases, most patients had minimal radiological involvement and were clinically less severe at presentation. These cases were also presumed to have a low mycobacterial burden, which is a known limitation affecting Xpert sensitivity. No technical errors were identified during testing (Table 3).

Table 3: Diagnostic Performance of XPERT MTB/RIF Compared to Culture (n=679)

Culture (Gold Standard)	Xpert Positive (Test +)	Xpert Negative (Test -)	Total
Positive (TB Present)	145	26	171
Negative (TB Absent)	18	490	508
Total	163	516	679

The diagnostic performance of the Xpert MTB/RIF test was evaluated against the culture gold standard. The test demonstrated a sensitivity of 84.8% (95% CI: 79.4–90.2%) and a specificity of 96.5% (95% CI: 94.8–98.1%). The PPV and NPV were 88.9% (95% CI: 84.1–93.7%) and 94.9% (95% CI: 93.0–96.8%), respectively. The overall diagnostic accuracy was 93.5% (95% CI: 91.7–95.4%) (Table 4).

Table 4: Diagnostic Performance Metrics of XPERT MTB/RIF

Metric	Calculation	Value (%)	95% CI
Sensitivity	$145 / (145 + 26) \times 100$	84.8	79.4% – 90.2%
Specificity	$490 / (18 + 490) \times 100$	96.5	94.8% – 98.1%
PPV	$145 / (145 + 18) \times 100$	88.9	84.1% – 93.7%
NPV	$490 / (26 + 490) \times 100$	94.9	93.0% – 96.8%
Diagnostic Accuracy	$(145 + 490) / 679 \times 100$	93.3	91.7% – 95.4%

Diagnostic accuracy was high across both gender and age groups, with no statistically significant differences. Males and females showed comparable sensitivity, specificity, and overall accuracy ($p=0.521$). Similarly, participants aged ≥ 40 years had slightly higher accuracy than those < 40 years, but the difference was not significant ($p=0.376$). Overall, the test performed consistently across all subgroups (Table 5).

Table 5: Diagnostic Accuracy Stratified by Gender

Variables	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	p-value
Gender	Male	85.7	95.8	89.5	93.5	0.521
	Female	83.3	97.4	88.1	96.3	
Age	< 40 Years	82.1	95.6	86.7	92.4	0.376
	≥ 40 Years	86.5	97.3	91.1	95.7	

Note: Chi-square was used to calculate the p-value. The p-value < 0.05 was statistically significant

The ROC analysis suggests that XPERT MTB/RIF has good discriminatory ability for the detection of tuberculosis when compared with culture. The estimated area under the curve (AUC) was 0.91, indicating a high true-positive rate

with a relatively low false-positive rate in this study population. These findings are consistent with the observed sensitivity (84.8%) and specificity (96.5%) of the test (Figure 1).

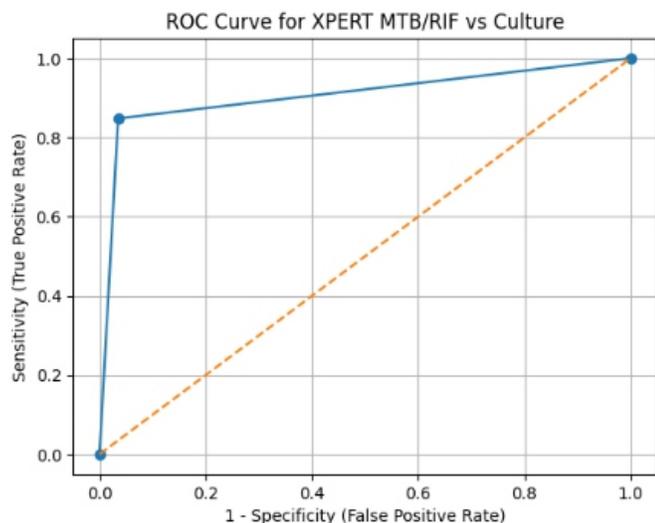


Figure 1: ROC Curve Analysis for Xpert MTB/RIF Versus Culture

DISCUSSION

In the current study, the diagnostic efficacy of Xpert MTB/RIF on BALF samples was compared using culture results as a reference. The results showed a higher sensitivity at 84.8%, specificity of 96.5%, PPV at 88.9%, NPV at 94.9%, and overall diagnostic accuracy of 93.3%. In a study carried out by Rahmati *et al.* in a Pakistani setting with a similar diagnostic objective using Xpert MTB/RIF on patients with smear-negative pulmonary TB, there was a comparable level of sensitivity at 84.5% with a higher specificity at 100% [9]. In a related study carried out on patients with a negligible sputum sample using BALF samples in a Pakistani setting, there was a comparable level of sensitivity at 85.3% with a higher specificity at 94.1% to underscore the diagnostic utility of Xpert [10]. A meta-analysis carried out by Mondoni *et al.* on the diagnostic accuracy of Xpert MTB/RIF on BAL samples revealed a pooled sensitivity and specificity of 87% and 92%, respectively, which closely resembled our values [11]. Xpert was found to have a sensitivity and specificity of 87.2% and 97.7%, respectively, with respect to BAL fluid in patients suspected of having pulmonary TB in a study carried out in China [12-14]. In a similar study carried out in India in patients with sputum scarce and smear-negative samples, Xpert was found to be 81.3% and 73.3% sensitive and specific, respectively, with culture as the reference standard [13]. A retrospective analysis carried out in Colombia revealed a high Xpert sensitivity of 91.7% and specificity of 90.1% using BAL samples [15, 16]. This small variation in both sensitivity and specificity can be explained by differences in sample size, TB prevalence,

subjects included in the studies, and processing of the samples. However, the high specificity and NPV in all studies affirm Xpert MTB/RIF's powerful rule-out test and its utility in a high burden of TB countries [17]. Sensitivity of 100% and specificity of 98.81% has been reported in earlier studies, outperforming traditional tests like Ziehl-Neelsen stain [18]. Also, in the current study, the high PPV of 88.9% further confirms the reliability of the test's negative results for not initiating any treatment, as evidenced by studies showing that high PPV aids in early initiation of treatment and successful control of TB infection [19]. Early diagnostic accuracy of tests translates into reduced transmission and improved outcomes, as evidenced by international statistics highlighting the importance of rapid diagnosis and subsequent treatment [20].

One of the key strengths of the current study is its large sample size and the use of BAL samples, which improves diagnostic accuracy in sputum-negative patients. In addition, assessing multiple diagnostic performance measures with culture as the gold standard adds to the reliability of the findings. Although Xpert MTB/RIF performed well in this cohort, its performance may vary across different settings, highlighting the need for ongoing evaluation and, in some cases, the use of complementary diagnostic tools for effective TB control.

CONCLUSIONS

This study's results confirm that Xpert MTB/RIF has high levels of accuracy regarding pulmonary TB in smear-negative patients when using BAL fluid samples. This level of performance is in line with both country and global studies, underpinning its importance as an initial testing methodology within resource-limited and endemic areas.

Authors' Contribution

Conceptualization: AS

Methodology: ZH, MAK

Formal analysis: MKS

Writing and Drafting: AS, ZI, FU

Review and Editing: AS, ZI, ZH, FU, MKS, MAK

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

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