Original Article

Diagnostic Value of Smear and Gene Xpert in Bronchoalveolar Lavage Fluid in Suspected Cases of Sputum Smear Negative Pulmonary Tuberculosis Patients

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Abstract

Background: Diagnosis of sputum smear-negative pulmonary tuberculosis (PTB) remains challenging because of the low bacillary burden and poor sensitivity of conventional smear microscopy. Bronchoalveolar lavage (BAL) obtained during bronchoscopy provides an alternative respiratory specimen for microbiological confirmation. The objective is to compare the diagnostic performance of BAL AFB smear and BAL CBNAAT using MGIT culture as the reference standard in patients with suspected sputum smear-negative pulmonary tuberculosis. **Material and Methods:** This hospital-based observational cross-sectional study included 100 adult patients with clinically and radiologically suspected sputum smear-negative PTB. BAL specimens obtained by flexible bronchoscopy were analyzed using AFB smear microscopy, CBNAAT, and MGIT culture. Diagnostic performance was assessed by calculating sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), diagnostic accuracy, and Cohen's kappa coefficient. **Results:** BAL CBNAAT detected Mycobacterium tuberculosis in 37.0% of patients, compared with 12.0% by BAL AFB smear, while MGIT culture confirmed tuberculosis in 33.0% of cases. BAL CBNAAT demonstrated significantly higher sensitivity (87.9% vs. 25.0%), NPV (93.7% vs. 72.7%), overall diagnostic accuracy (88.0% vs. 73.5%), and stronger agreement with MGIT culture (κ=0.737 vs. 0.267) than BAL AFB smear. Although BAL AFB smear showed higher specificity (97.0% vs. 88.1%), its low sensitivity limited its diagnostic utility. **Conclusion:** BAL CBNAAT demonstrated superior diagnostic performance over BAL AFB smear for the diagnosis of suspected sputum smear-negative pulmonary tuberculosis. Its high sensitivity and diagnostic accuracy support its routine use as the preferred microbiological investigation in patients undergoing bronchoscopy, particularly when combined with mycobacterial culture for definitive confirmation.

Keywords: Pulmonary tuberculosis, Bronchoalveolar lavage, GeneXpert MTB/RIF, CBNAAT, Acid-fast bacilli smear, Mycobacterial culture.

Received: 10 August 2026 **Revised: 20 August 2026** **Accepted: 02 September 2026** **Published: 12 September 2026**

Introduction

Tuberculosis (TB) remains one of the leading causes of death from a single infectious agent worldwide.[1,2] According to the World Health Organization (WHO) Global Tuberculosis Report 2024, an estimated 10.8 million people developed tuberculosis and approximately 1.25 million died from the disease in 2023, with India accounting for nearly 26% of the global TB burden, highlighting the continued public health challenge posed by the disease.[1,3]

Pulmonary tuberculosis (PTB) is the most common form of tuberculosis and the principal source of disease transmission. Early microbiological confirmation is essential for prompt initiation of anti-tubercular therapy, reduction of disease transmission, and improvement of clinical outcomes.[4,5] However, establishing the diagnosis remains particularly challenging in patients with sputum smear-negative pulmonary tuberculosis (SNPT), who often have a low bacillary load, early-stage disease, or are unable to produce adequate sputum.[6,7] Consequently, a substantial proportion of clinically suspected PTB cases remain microbiologically unconfirmed despite suggestive clinical and radiological findings, leading to delayed diagnosis and treatment.[8,9]

Flexible fibre-optic bronchoscopy with bronchoalveolar lavage

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DOI: 10.21276/amit.2026.v13.i3.1033

How to cite this article: Gandhi H, Kumar A, Nirala P, Maqusood M, Jethi C, Chaplot C, Goel A, Rajak M. Diagnostic value of smear and Gene Xpert in Bronchoalveolar Lavage fluid in Suspected cases of Sputum Smear Negative Pulmonary Tuberculosis Patients. Acta Med Int. 2026;13(3):148-153.

(BAL) has emerged as an important diagnostic tool in smear-negative PTB by allowing direct sampling from the affected pulmonary segments.[10] BAL specimens can be analyzed by acid-fast bacilli (AFB) smear microscopy, cartridge-based nucleic acid amplification testing (CBNAAT/GeneXpert), and mycobacterial culture.[11] While BAL AFB smear provides rapid preliminary evidence of tuberculosis, its sensitivity is limited in paucibacillary disease. In contrast, BAL CBNAAT enables rapid detection of Mycobacterium tuberculosis with simultaneous identification of rifampicin resistance, whereas BAL mycobacterial culture remains the reference standard because of its superior sensitivity and ability to perform drug susceptibility testing.[12,13]

Therefore, the present study was undertaken to evaluate and compare the diagnostic performance of BAL AFB smear, BAL CBNAAT (GeneXpert), and BAL mycobacterial culture in patients with suspected sputum smear-negative pulmonary tuberculosis, with the aim of identifying the most effective microbiological approach for early and accurate diagnosis.

Materials and Methods

Study Design and Setting: This hospital-based observational cross-sectional study was conducted in the Department of Respiratory Medicine, Teerthanker Mahaveer Medical College & Research Centre (TMMC&RC), Moradabad, Uttar Pradesh, India, over a period of 18 months after obtaining approval from the Institutional Ethics Committee (IEC). The study included adult patients with clinically suspected pulmonary tuberculosis who were sputum smear-negative or unable to produce sputum. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki, and written informed consent was obtained from all participants prior to enrolment.

Sample Size Estimation:

The sample size was calculated using the formula:

$n =$

$$n = \frac{Z^2_{\alpha/2} \times Sp(1-Sp)}{(1-P) \times d^2}$$

where Zα/2 = 1.96 at a 95% confidence interval, specificity (Sp) = 93%, prevalence (P) = 30%, and absolute precision (d) = 6%. Based on these assumptions, the minimum required sample size was 99 participants, all of whom were enrolled in the study.

Study Participants: Patients aged >18 years presenting with clinical and radiological features suggestive of pulmonary tuberculosis and having sputum smear-negative microscopy or inability to expectorate sputum were screened for eligibility. Patients already receiving anti-tubercular treatment, those with extrapulmonary tuberculosis, or those with contraindications to bronchoscopy were excluded. Consecutive eligible patients fulfilling the inclusion and exclusion criteria were recruited during the study period.

Data Collection and Diagnostic Procedures: Demographic characteristics, clinical history, presenting symptoms, radiological findings, and laboratory investigations were recorded using a standardized case record form. All participants underwent chest radiography, and computed tomography (CT) of the thorax was performed whenever clinically indicated. Flexible fibre-optic bronchoscopy was carried out under standard aseptic precautions, and bronchoalveolar lavage (BAL) fluid was collected from the radiologically involved lung segment.

BAL specimens were processed for Ziehl–Neelsen (ZN) staining for acid-fast bacilli (AFB) smear microscopy, cartridge-based nucleic acid amplification testing (CBNAAT/GeneXpert MTB/RIF), and mycobacterial culture. BAL mycobacterial culture was considered the reference standard for microbiological confirmation of pulmonary tuberculosis. The diagnostic performance of BAL AFB smear and BAL CBNAAT was assessed by comparison with BAL culture.

Outcome Measures: The primary outcome of the study was the microbiological diagnosis of pulmonary tuberculosis using BAL specimens. The diagnostic performance of BAL AFB smear and BAL CBNAAT was evaluated against BAL mycobacterial culture by determining sensitivity, specificity, positive predictive value, negative predictive value, and overall diagnostic accuracy.

Statistical Analysis: Data were entered into Microsoft Excel and analyzed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD) or median (interquartile range [IQR]), and categorical variables as frequencies and percentages. Diagnostic performance was evaluated using sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and Cohen's kappa coefficient. A p value <0.05 was considered statistically significant.

Results

A total of 100 patients with clinically and radiologically suspected sputum smear-negative pulmonary tuberculosis were included in the study. The mean age was 50.8 ± 17.6 years, with a predominance of males (66.0%). Most participants belonged to the 46–60-year age group (38.0%) and were from rural areas (74.0%). Cough was the most common presenting symptom (85.0%), followed by fever (56.0%), whereas hemoptysis was reported in 22.0% of patients. Baseline demographic and clinical characteristics are summarized in [Table 1]

Table 1: Baseline demographic and clinical characteristics of the study participants

Variable	n (%) / Mean ± SD
Age (years)	
Mean ± SD	50.8 ± 17.6
Median (IQR)	54.0 (38.3–63.0)
18–30	19 (19.0)

31–45	14 (14.0)
46–60	38 (38.0)
>60	29 (29.0)
Sex	
Male	66 (66.0)
Female	34 (34.0)
Residence	
Rural	74 (74.0)
Urban	26 (26.0)
Presenting symptoms*	
Cough	85 (85.0)
Fever	56 (56.0)
Chest pain	34 (34.0)
Shortness of breath	34 (34.0)
Hemoptysis	22 (22.0)
Loss of appetite	16 (16.0)

Microbiological evaluation of bronchoalveolar lavage (BAL) specimens demonstrated marked differences among the three diagnostic modalities. BAL AFB smear was positive in 12.0% of patients, whereas BAL CBNAAT detected Mycobacterium tuberculosis in 37.0% of cases. MGIT culture confirmed tuberculosis in 33.0% of patients, while 6.0% of cultures yielded non-tuberculous mycobacteria (NTM). These findings indicate a substantially higher detection rate of BAL CBNAAT compared with conventional BAL AFB smear [Table 2]

Table 2: Microbiological Test Results of Bronchoalveolar Lavage (BAL) Samples:

Symptoms	Frequency	Percentage
BAL AFB		
Positive (1+)	7	7.0
Positive (2+)	2	2.0
Positive (3+)	1	1.0
Scanty	2	2.0
Negative	88	88.0
BAL CBNAAT		
Positive	37	37.0
Negative	63	63.0
MGIT Culture		
Positive	27	27.0
Negative	67	67.0
NTM	6	6.0

Using MGIT culture as the reference standard, BAL CBNAAT correctly identified 29 of 33 culture-positive patients (87.9%), while only four culture-positive cases were missed. Among culture-negative patients, BAL CBNAAT correctly classified 59 of 67 (88.1%), with eight false-positive results. The agreement between BAL CBNAAT and MGIT culture was statistically significant ($\kappa = 0.737$, $p < 0.0001$), indicating substantial diagnostic concordance [Table 3]

Table 3: Diagnostic performance of BAL CBNAAT compared with MGIT culture

BAL CBNAAT	MGIT Culture (Gold Standard)		p-value
	Positive	Negative	
Positive	29(87.9%)	8(11.9%)	<0.0001
Negative	4(12.1%)	59(88.1%)	
Total	33(100.0%)	67(100.0%)	
k=0.737, p<0.0001			
Parameter		Value	
Sensitivity		87.90%	
Specificity		88.10%	
PPV		78.40%	
NPV		93.70%	
Accuracy		88.00%	
Cohen's κ		0.737	

In comparison, BAL AFB smear detected only 8 of 32 culture-positive cases (25.0%), missing three-fourths of microbiologically confirmed tuberculosis cases. Although BAL AFB smear demonstrated high specificity (97.0%), its agreement with MGIT culture was only fair ($\kappa = 0.267$, $p = 0.001$), reflecting its limited sensitivity in sputum smear-negative pulmonary tuberculosis [Table 4]

Table 4: Diagnostic performance of BAL AFB smear compared with MGIT culture

BAL AFB	MGIT Culture (Gold Standard)		p-value
	Positive	Negative	
Positive	8(25.0%)	2(3.0%)	0.002
Negative	24(75.0%)	64(97.0%)	
Total	32(100.0%)	66(100.0%)	
k=0.267, p=0.001			
Parameter	Value		
Sensitivity	25.00%		
Specificity	97.00%		
PPV	80.00%		
NPV	72.70%		
Accuracy	73.50%		
Cohen's κ	0.267		

Direct comparison of diagnostic performance showed that BAL CBNAAT markedly outperformed BAL AFB smear. BAL CBNAAT demonstrated a sensitivity of 87.9%, specificity of 88.1%, positive predictive value of 78.4%, negative predictive value of 93.7%, and overall diagnostic accuracy of 88.0%. In contrast, BAL AFB smear showed a sensitivity of only 25.0%, despite a slightly higher specificity (97.0%). Overall diagnostic accuracy was considerably lower for BAL AFB smear (73.5%) than for BAL CBNAAT (88.0%) [Table 5 and Figure 1] These findings highlight the superior diagnostic utility of BAL CBNAAT for the early microbiological diagnosis of sputum smear-negative pulmonary tuberculosis.

Table 5: Comparison of diagnostic performance of BAL CBNAAT and BAL AFB smear

Diagnostic parameter	BAL CBNAAT	BAL AFB smear
Sensitivity (%)	87.9	25
Specificity (%)	88.1	97
Positive predictive value (%)	78.4	80
Negative predictive value (%)	93.7	72.7
Overall accuracy (%)	88	73.5
Cohen's κ	0.737	0.267
Agreement	Substantial	Fair

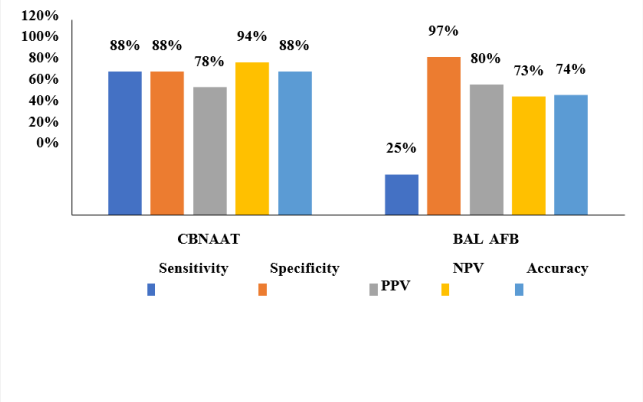

Figure 1: Comparison of Sensitivity, Specificity, PPV, NPV and Accuracy of BAL CBNAAT and BAL AFB Smear

Discussion

The present study evaluated the diagnostic performance of bronchoalveolar lavage (BAL) acid-fast bacilli (AFB) smear and cartridge-based nucleic acid amplification test (CBNAAT) in patients with clinically and radiologically suspected sputum smear-negative pulmonary tuberculosis (PTB), using MGIT culture as the microbiological reference standard. A total of 100 patients were included in the study. BAL CBNAAT demonstrated a significantly higher microbiological detection rate than BAL AFB smear (37.0% vs. 12.0%) and exhibited superior sensitivity (87.9%), negative predictive value (93.7%) and overall diagnostic accuracy (88.0%), whereas BAL AFB smear showed high specificity (97.0%) but poor sensitivity (25.0%). These findings emphasize the greater diagnostic utility of BAL CBNAAT for early microbiological confirmation of smear-negative PTB.

The demographic profile of the present study demonstrated a mean age of 50.8 ± 17.6 years, with male predominance (66%) and a higher proportion of rural residents (74%). Cough and fever were the most frequent presenting symptoms. Similar demographic characteristics have been reported by Johar et al,[14] who observed that smear-negative pulmonary tuberculosis predominantly affected young and middle-aged males and was frequently associated with cough and constitutional symptoms. Likewise, Iyer et al,[15] reported a male predominance among patients undergoing bronchoscopy for suspected smear-negative PTB, suggesting that delayed diagnosis is more common in economically productive age groups because of prolonged exposure and healthcare-seeking behavior. Although demographic characteristics are useful in describing the study population, previous studies, including ours, indicate that they have limited value in differentiating microbiologically confirmed tuberculosis from other pulmonary diseases.

Our microbiological findings demonstrated that BAL CBNAAT detected 37.0% of tuberculosis cases compared with 12.0% by BAL AFB smear, while MGIT culture confirmed tuberculosis in 33.0% of patients. These observations are consistent with Mishra et al,[16] who reported that BAL CBNAAT achieved the highest microbiological yield among bronchoscopic investigations, whereas BAL smear showed the lowest detection

rate. Similarly, Dubey et al,[17] demonstrated that BAL CBNAAT substantially improved bacteriological confirmation compared with conventional smear microscopy and additionally enabled early detection of rifampicin resistance. Musso et al,[18] further reported that bronchoscopy-guided BAL achieved a markedly higher diagnostic yield than induced sputum, supporting the role of BAL as the preferred respiratory specimen in sputum smear-negative patients. Collectively, these studies reinforce our findings that BAL CBNAAT significantly enhances microbiological diagnosis in patients with low bacillary burden.

When BAL CBNAAT was evaluated against MGIT culture, the present study demonstrated a sensitivity of 87.9%, specificity of 88.1%, and substantial agreement with culture ($\kappa = 0.737$). These findings closely resemble those reported by Uddin et al,[19] who observed that Xpert MTB/RIF possessed the highest sensitivity among BAL-based diagnostic techniques and exhibited superior discriminatory ability compared with smear microscopy and culture. Similarly, Abdel Wahab Koraa et al,[20] concluded that GeneXpert performed on BAL specimens produced diagnostic results comparable to culture while substantially shortening the time to diagnosis. Archana B et al,[21] likewise demonstrated high sensitivity of BAL CBNAAT relative to culture and recommended its routine use for rapid confirmation of smear-negative pulmonary tuberculosis. The high negative predictive value observed in our study further suggests that BAL CBNAAT is particularly valuable for excluding active tuberculosis in clinically suspected patients.

In contrast, BAL AFB smear demonstrated only 25.0% sensitivity, despite maintaining a specificity of 97.0% and a positive predictive value of 80.0%. Furthermore, the agreement with MGIT culture was only fair ($\kappa = 0.267$), indicating that smear microscopy alone misses a considerable proportion of microbiologically confirmed tuberculosis cases. Similar findings have been reported by Novin et al,[22] who concluded that smear microscopy consistently underperformed compared with molecular and culture-based techniques in sputum-negative patients. Likewise, Bachh et al,[23] demonstrated that BAL culture yielded significantly more confirmed tuberculosis cases than BAL smear microscopy, emphasizing that smear examination should not be relied upon as the sole microbiological investigation in suspected smear-negative PTB. These observations are consistent with the inherently low bacillary burden encountered in smear-negative disease, which limits the sensitivity of direct microscopy.

Direct comparison of both diagnostic modalities clearly demonstrated the superiority of BAL CBNAAT over BAL AFB smear. Although BAL AFB smear showed marginally higher specificity (97.0% vs. 88.1%), BAL CBNAAT exhibited markedly better sensitivity (87.9% vs. 25.0%), higher negative predictive value (93.7% vs. 72.7%), greater overall diagnostic accuracy (88.0% vs. 73.5%) and substantially stronger agreement with MGIT culture. Similar conclusions were drawn by Dubey et al,[17] Archana B et al,[21] and Musso et al,[18] all of whom demonstrated that BAL-based molecular testing provides significantly greater diagnostic yield than conventional smear microscopy in sputum smear-negative pulmonary tuberculosis. The consistency of these findings across different populations supports the growing evidence that molecular testing should be prioritized whenever bronchoscopy is performed for suspected smear-negative PTB, while BAL AFB smear should be regarded as an adjunctive rather than definitive diagnostic investigation.

Conclusion

Bronchoalveolar lavage (BAL) CBNAAT demonstrated significantly superior diagnostic performance compared with BAL AFB smear in patients with suspected sputum smear-negative pulmonary tuberculosis. BAL CBNAAT showed markedly higher sensitivity, negative predictive value, overall diagnostic accuracy and stronger agreement with MGIT culture, while BAL AFB smear exhibited high specificity but limited sensitivity. These findings suggest that BAL CBNAAT should be considered the preferred microbiological investigation in patients undergoing bronchoscopy for suspected smear-negative PTB. When combined with mycobacterial culture, BAL CBNAAT facilitates earlier and more accurate diagnosis, enabling timely initiation of anti-tubercular therapy and improving patient management.

Recommendation: BAL CBNAAT should be considered the preferred initial diagnostic modality in patients with suspected sputum smear-negative pulmonary tuberculosis undergoing bronchoscopy because of its superior sensitivity and diagnostic accuracy. Routine use of BAL CBNAAT in combination with mycobacterial culture may facilitate earlier microbiological confirmation, timely initiation of anti-tubercular therapy and improved patient outcomes. Further multicentre studies with larger sample sizes are recommended to validate these findings and assess their generalizability.

Limitation: The present study was conducted at a single tertiary care center with a relatively small sample size, which may limit the generalizability of the findings. In addition, only bronchoalveolar lavage specimens were evaluated and long-term treatment outcomes were not assessed. Future multicenter prospective studies involving larger populations and follow-up data are warranted to further establish the diagnostic utility of BAL CBNAAT in sputum smear-negative pulmonary tuberculosis.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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