

Original Article

Combined molecular assays and MGIT culture in bronchoalveolar lavage fluid for smear-negative, newly diagnosed pulmonary tuberculosis

Wenbin Liu, Chengsong Cai, Leiming Tang, Feng Pan

Department of Laboratory Medicine, The Affiliated Hospital of Hangzhou Normal University, Hangzhou 310015, Zhejiang, China

Received April 16, 2026; Accepted August 27, 2026; Epub October 15, 2026; Published October 30, 2026

Abstract: Objective: To evaluate the diagnostic performance of combined testing strategies using Xpert MTB/RIF (Xpert), TB-DNA, and MGIT culture in patients with smear-negative, newly diagnosed pulmonary tuberculosis (PTB) and to determine the optimal strategy for early diagnosis. Methods: In this retrospective observational study, 288 inpatients were enrolled from July 2022 to November 2023. Using bronchoalveolar lavage fluid (BALF) samples, we evaluated the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), diagnostic accuracy, kappa value, and the area under the curve (AUC) of Xpert, TB-DNA, and MGIT culture, individually and in combination. Results: With MGIT culture as the reference standard, only specificity differed significantly between Xpert and TB-DNA ($P < 0.05$). No significant differences were observed in sensitivity, PPV, NPV, or diagnostic accuracy. With the final clinical diagnosis as the reference standard, only sensitivity differed significantly between the two assays ($P < 0.05$). Among the four combination strategies, the triple-method assay achieved the highest sensitivity (81.69%; 95% CI: 74.52-87.19), NPV (76.15%; 95% CI: 67.34-83.17), diagnostic accuracy (85.41%; 95% CI: 80.30-89.37), AUC (0.864; 95% CI: 0.814-0.915), and kappa value (0.704). However, its sensitivity did not differ significantly from that of the combinations of Xpert with TB-DNA or TB-DNA with MGIT culture (both $P > 0.05$). No significant differences were observed among the four strategies in specificity, PPV, NPV, or diagnostic accuracy (all $P > 0.05$). All four combination strategies showed substantial agreement with the final clinical diagnosis, and their AUC values exceeded 0.83. Conclusion: In BALF samples from patients with smear-negative, newly diagnosed PTB, Xpert and TB-DNA showed similar overall diagnostic performance. The combinations of Xpert with TB-DNA and TB-DNA with MGIT culture showed diagnostic performance comparable to that of the triple-method combination. The triple-method combination, which integrates two molecular assays with MGIT culture, achieved the highest sensitivity and diagnostic accuracy. This strategy may therefore improve early diagnostic assessment and support treatment decisions in this patient population.

Keywords: Tuberculosis, Xpert MTB/RIF, TB-DNA, MGIT culture, bronchoalveolar lavage fluid

Introduction

Pulmonary tuberculosis (PTB) is the most common form of tuberculosis and remains a major global public health challenge. It is caused by the *Mycobacterium tuberculosis* (MTB) complex. According to the World Health Organization 2025 Global Tuberculosis Report, 8.3 million newly diagnosed tuberculosis cases were reported globally in 2024, representing a slight increase from 8.2 million in 2023. Tuberculosis caused an estimated 1.23 million deaths globally, approximately twice the number attributed

to human immunodeficiency virus/acquired immunodeficiency syndrome, with a case fatality rate of 11.5% [1]. Smear-negative PTB remains particularly difficult to diagnose, often delaying treatment initiation. Improved diagnostic strategies, including combined testing of bronchoalveolar lavage fluid (BALF) samples, are therefore needed to facilitate earlier and more reliable diagnosis.

Traditional acid-fast bacilli (AFB) smear microscopy remains widely used because of its low cost and operational simplicity; however, its

sensitivity is relatively low, with single-slide positivity rates ranging from 22% to 43% [2]. MGIT culture remains an important microbiological reference method because of its higher sensitivity than AFB smear microscopy. However, its clinical utility is constrained by the prolonged time required for mycobacterial growth [3, 4]. Xpert MTB/RIF (Xpert) is an automated nucleic acid amplification platform that addresses some of these gaps by rapidly identifying MTB and rifampicin resistance within 2 hours. However, similar to other molecular assays, it has an important limitation: it cannot distinguish among viable bacteria, dormant bacilli, and nonviable but structurally intact MTB released during effective anti-tuberculosis treatment. Consequently, it falls short as a tool for monitoring treatment response or supplanting conventional AFB smear and culture methods [4]. The TB-DNA assay uses TaqMan probe-based polymerase chain reaction (PCR), with primers and probes targeting conserved sequences of the *Mycobacterium* genus and MTB for qualitative detection of mycobacterial nucleic acids in clinical samples. This design enables differentiation of MTB from nontuberculous mycobacteria (NTM). However, like Xpert, TB-DNA cannot distinguish viable from nonviable MTB and is therefore unsuitable for monitoring treatment response [5]. Moreover, no single assay provides sufficient sensitivity across all clinical settings [6]. Combining molecular assays with culture may therefore improve diagnostic yield while retaining the ability to detect viable organisms, providing a rationale for evaluating combined testing strategies in smear-negative PTB.

Previous studies have confirmed that, compared with sputum samples, BALF samples can provide a higher diagnostic yield for PTB, particularly when conventional respiratory samples are negative or inadequate [7, 8]. BALF is particularly valuable for diagnosing smear-negative PTB because it can improve microbiological detection compared with conventional sputum testing [9]. Approximately 54% of smear-negative PTB cases are ultimately confirmed via nucleic acid amplification testing or culture of BALF samples [10]. Although fiberoptic bronchoscopy is invasive, BALF testing can increase the rate of microbiological confirmation in patients with bacteriologically negative, non-productive, or scant-sputum PTB [11, 12].

Accordingly, this study aimed to evaluate the diagnostic performance of Xpert, TB-DNA, and MGIT culture, individually and in combination, using BALF samples from patients with smear-negative, newly diagnosed PTB. The findings may inform the selection of testing strategies for earlier and more accurate diagnosis in this population.

Data and methods

Patient selection

This retrospective observational study included 288 inpatients at the Affiliated Hospital of Hangzhou Normal University, a designated tuberculosis referral center, from July 2022 to November 2023. No participant had prior anti-tuberculosis treatment. Therefore, all confirmed PTB cases were newly diagnosed. BALF samples were collected from all enrolled patients, and tested using AFB smear microscopy, Xpert, TB-DNA, and MGIT culture. We recorded all test results for analysis. Trained laboratory staff performed all assays. Every procedure followed the manufacturers' instructions and relevant industry standards. To ensure accurate and reliable results, we conducted routine internal quality control and external quality assessment. We evaluated sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy using clinical diagnosis or MGIT culture as the reference standard. The clinical diagnosis of PTB followed the national guidelines issued by the National Health and Family Planning Commission in China [13], which integrate evidence from clinical presentation, imaging features, and microbiological, immunological, and pathological findings. This study was approved by the Medical Ethics Committee of the Affiliated Hospital of Hangzhou Normal University, with approval number 2026 (E2)-KS-020.

Eligibility criteria

The inclusion criteria were as follows: (1) BALF samples had a volume of ≥ 5 mL; (2) AFB smear microscopy, Xpert, TB-DNA, and MGIT culture were performed using the same BALF sample; (3) complete clinical diagnostic data were available; and (4) patients had no history of tuberculosis or prior anti-tuberculosis treatment.

The exclusion criteria were as follows: (1) confirmed infection with NTM; (2) positive Xpert or TB-DNA results with lost outpatient follow-up records; and (3) patients who had contraindications to bronchoscopy.

Instruments and methods

(1) AFB smear microscopy: AFB smear microscopy was performed using the Ziehl-Neelsen staining method (Baso, China). The procedure followed the national standard for the diagnosis of pulmonary tuberculosis (WS 288-2017) [13].

(2) Xpert assay: The Xpert MTB/RIF assay (Cepheid, USA) and its reagent kit were used. BALF was mixed with pretreatment reagent in a 1:2 ratio, vortexed, and allowed to stand for 15 min. The processed sample was then transferred to an Xpert cartridge and loaded into the instrument for automated analysis and result reporting.

(3) TB-DNA assay: A *Mycobacterium* nucleic acid detection kit (CapitalBio, China) and an MA-6000 fluorescent quantitative PCR instrument (Molarray, China) were used. Testing was performed according to the manufacturer's instructions.

(4) MGIT culture: MGIT culture was performed via the BACTEC MGIT 960 system (BD, USA). Briefly, 2-5 mL of BALF was mixed thoroughly with 1-2 volumes of NALC-NaOH (N-acetyl-L-cysteine-sodium hydroxide) to decontaminate and liquefy the sample. After standing at room temperature for 15 min, the mixture was supplemented with 45 mL of phosphate-buffered saline (PBS) to the final volume, followed by centrifugation at 3000×g for 15-20 min. The supernatant was discarded, and the pellet was resuspended in 1 mL of PBS and vortexed vigorously. Subsequently, 0.5 mL of the processed sediment was inoculated into an MGIT tube supplemented with 0.8 mL of MGIT growth supplement, mixed by inversion, and incubated in the BACTEC MGIT 960 system. MGIT tubes with positive growth signals were subjected to AFB smear microscopy and MPT64 antigen testing for species identification. AFB smear-positive, MPT64-negative cultures were further evaluated using the Xpert assay.

Statistical analysis

Statistical analyses were conducted using SPSS Statistics version 31.0. Continuous variables were expressed as mean ± standard deviation (SD), whereas categorical variables were expressed as frequencies and percentages [n (%)]. Categorical variables were compared between groups using Pearson's chi-square test or Fisher's exact test, as appropriate. Diagnostic performance measures, including sensitivity, specificity, PPV, NPV, and diagnostic accuracy, together with their corresponding 95% confidence intervals (CIs), were calculated using OpenEpi, version 3, an online statistical software package. Receiver operating characteristic (ROC) curves were constructed for each diagnostic assay, and the area under the curve (AUC) and corresponding 95% CI were calculated to assess diagnostic discrimination. Agreement with the reference diagnosis was assessed using kappa (κ) statistics. Kappa values were interpreted according to the criteria of Landis and Koch: poor agreement (κ ≤ 0), slight agreement (0 < κ ≤ 0.20), fair agreement (0.20 < κ ≤ 0.40), moderate agreement (0.40 < κ ≤ 0.60), substantial agreement (0.60 < κ ≤ 0.80), and almost perfect agreement (0.80 < κ ≤ 1.00). All the statistical tests were two-sided, and *P* values < 0.05 were considered statistically significant.

Results

Characteristics of the study population

As shown in the flow chart (**Figure 1**), we retrospectively screened 288 inpatients at the Affiliated Hospital of Hangzhou Normal University from July 2022 to November 2023. Baseline demographic and clinical characteristics of the study population are summarized in **Table 1**. Patient age ranged from 13 to 85 years, with a mean of 45.30 ± 17.88 years. 185 patients (64.24%) were male, and 103 (35.76%) were female. We classified all patients into three mutually exclusive diagnostic categories based on AFB smear results and final clinical diagnoses. Specifically, 142 patients (49.31%) were confirmed to have smear-negative PTB. 55 patients (19.10%) had smear-positive PTB. The remaining 91 patients (31.60%) were diagnosed with non-PTB conditions, including pneumonia, other non-tuber-

Molecular assays and MGIT for newly diagnosed PTB

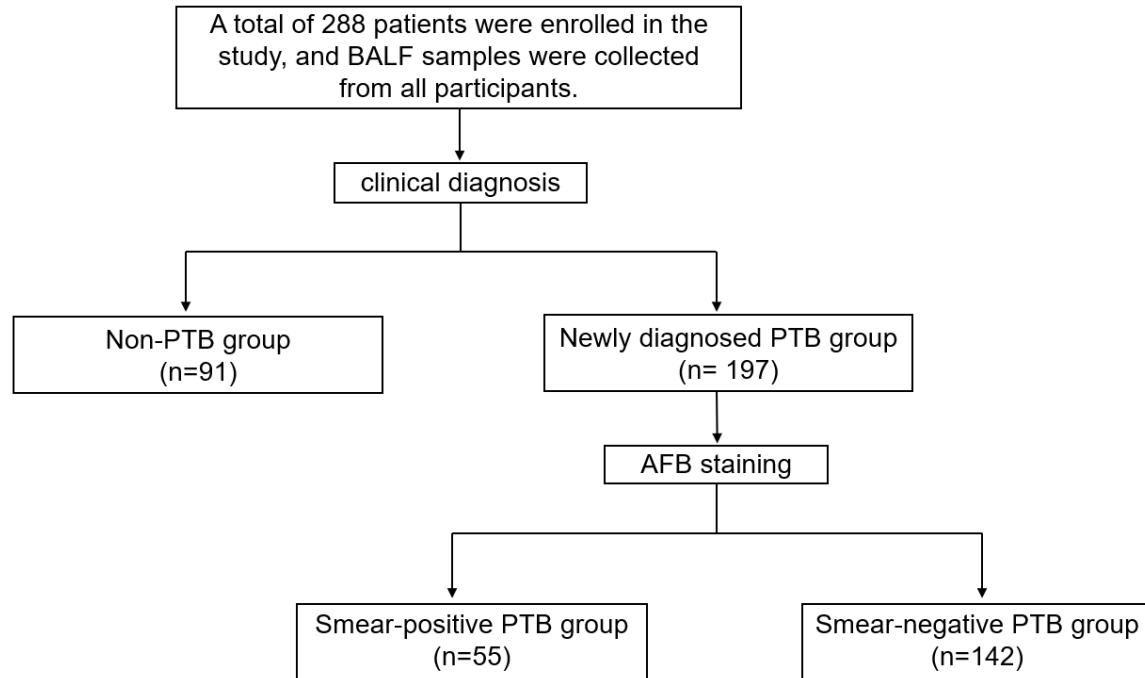


Figure 1. Patient grouping flow chart. BALF, bronchoalveolar lavage fluid; PTB, pulmonary tuberculosis; AFB, acid-fast bacilli.

Table 1. Demographic and clinical characteristics of the study population

Characteristics	Patients (n = 288)
Age (years)	45.30 ± 17.88
Gender (n, %)	
Male	185 (64.24)
Female	103 (35.76)
Previous tuberculosis treatment (n, %)	
Yes	0 (0)
No	288 (100)
Clinical diagnosis (n, %)	
Smear-positive PTB	55 (19.10)
Smear-negative PTB	142 (49.31)
Non-PTB	91 (31.60)
AFB smear (n, %)	
MTB positive	55 (19.10)
MTB negative	233 (80.90)
Xpert (n, %)	
MTB positive	143 (49.65)
MTB negative	145 (50.35)
TB-DNA (n, %)	
MTB positive	165 (57.29)
MTB negative	123 (42.71)
MGIT culture (n, %)	
MTB positive	111 (38.54)
MTB negative	177 (61.46)

PTB, pulmonary tuberculosis; AFB, acid-fast bacilli; Xpert, Xpert MTB/RIF; MTB, *Mycobacterium tuberculosis*.

culous pulmonary infections, and pulmonary opacities.

AFB smear results in all patients

Among the 197 PTB patients, 55 (27.92%) were AFB smear positive, 142 (72.08%) were smear negative, and all 91 (100%) non-PTB patients had negative AFB smear results. When clinical diagnosis was used as the standard, the AFB smear had a sensitivity of 27.92% (95% CI: 22.12-34.56), a specificity of 100% (95% CI: 95.95-100), a PPV of 100% (95% CI: 93.47-100), an NPV of 39.06% (95% CI: 33.02-45.45), and a diagnostic accuracy of 50.69% (95% CI: 44.95-56.42). The AFB smear-positive images are shown in **Figure 2A**. As shown in **Table 2**, the AFB smear results for the BALF samples from newly diagnosed PTB patients were in slight agreement with the clinical diagnosis (kappa = 0.197, $P < 0.001$).

Molecular assays and MGIT for newly diagnosed PTB

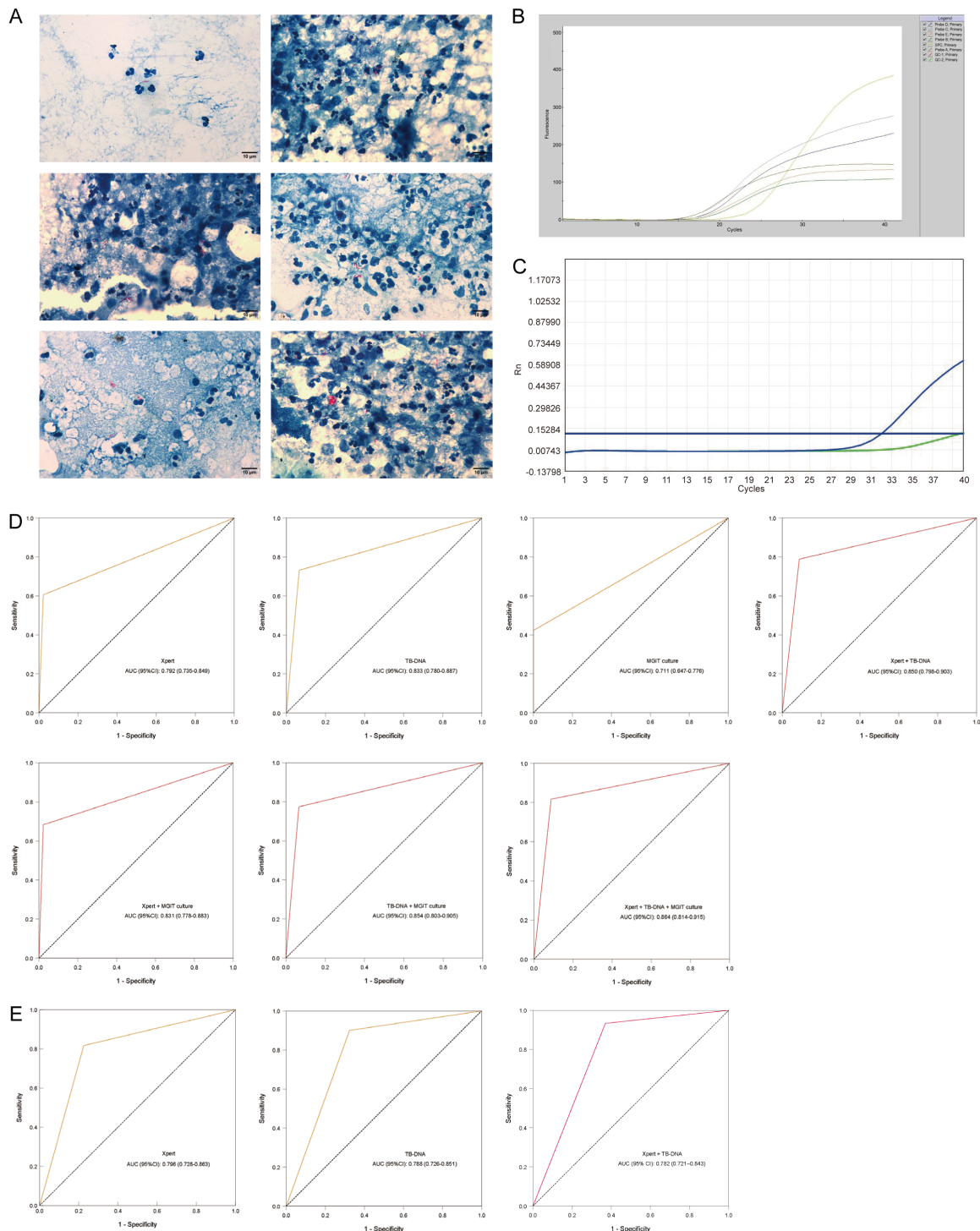


Figure 2. A. Representative images depicting AFB smear-positive results from BALF samples from newly diagnosed PTB patients stained via the Ziehl-Neelsen method. Magnification, $\times 1,000$. B. Representative Xpert assay result from a BALF sample from a smear-negative, newly diagnosed PTB patient. The amplification plot indicates a positive MTB result with rifampicin susceptibility. C. Representative amplification plot of the TB-DNA assay. The data were derived from a BALF sample from a smear-negative, newly diagnosed PTB patient, indicating a positive MTB result. The x-axis represents the number of PCR cycles, and the y-axis represents the fluorescence intensity (Rn). D. ROC curves of Xpert, TB-DNA, MGIT culture and their combination, with clinical diagnosis used as a reference standard for detecting PTB. E. ROC curves of Xpert, TB-DNA, and their combination, with MGIT culture used as a reference standard for detecting PTB. AFB, acid-fast bacilli; BALF, bronchoalveolar lavage fluid; PTB, pulmonary tuberculosis; Xpert, Xpert MTB/RIF; MGIT, MGIT culture; MTB, *Mycobacterium tuberculosis*; PCR, polymerase chain reaction; ROC, receiver operating characteristic; AUC, area under the curve.

Molecular assays and MGIT for newly diagnosed PTB

Table 2. AFB smear results and agreement with clinical diagnoses in BALF samples from the PTB and non-PTB groups

AFB smear	Total (n = 288)	PTB (n = 197)	Non-PTB (n = 91)	Kappa value	P value
MTB positive	55	55	0	0.197	< 0.001
MTB negative	233	142	91		

BALF, bronchoalveolar lavage fluid; PTB, pulmonary tuberculosis; AFB, acid-fast bacilli; MTB, *Mycobacterium tuberculosis*.

Table 3. Positivity rates of Xpert, TB-DNA, and MGIT culture in BALF samples from smear-negative and smear-positive PTB groups

Diagnostic assay	SP-PTB			SN-PTB			χ^2	P value
	MTB positive	MTB negative	PR (%)	MTB positive	MTB negative	PR (%)		
Xpert	55	0	100	86	56	60.56	-	< 0.001
TB-DNA	55	0	100	104	38	73.24	-	< 0.001
MGIT	51	4	92.73	60	82	42.25	39.034	< 0.001

BALF, bronchoalveolar lavage fluid; PR, positivity rate; PTB, pulmonary tuberculosis; SP-PTB, smear-positive PTB; SN-PTB, smear-negative PTB; Xpert, Xpert MTB/RIF; MGIT, MGIT culture; MTB, *Mycobacterium tuberculosis*.

Comparison of positivity rates of the single-method assays using clinical diagnosis as the reference standard between smear-negative and smear-positive PTB groups

Xpert utilizes five probes specifically designed to target the rifampicin resistance-determining region of the *rpoB* gene and integrates multiple internal quality control measures. **Figure 2B** illustrates representative amplification curves. Primers and probes targeting sequences specific to *Mycobacterium* and MTB are used in the TB-DNA assay. A positive MTB result requires a typical sigmoidal amplification curve specific to MTB and a cycle threshold (Ct) value < 40. Representative results are depicted in **Figure 2C**. In the 55 AFB smear-positive PTB patients, positive results were obtained via Xpert, TB-DNA, and MGIT culture in 55 (100%), 55 (100%), and 51 (92.73%) patients, respectively. Among the 142 smear-negative cases, 86 (60.56%), 104 (73.24%), and 60 (42.25%) were positive. The positivity rates of Xpert, TB-DNA, and MGIT culture in BALF samples were compared between the smear-negative and smear-positive PTB groups (**Table 3**), with the clinical diagnosis serving as the reference standard. The findings showed that the positivity rates for all three methods were significantly higher in the smear-positive PTB group than in the smear-negative PTB group. The positivity rates for all three single methods differed significantly between the two groups (all $P < 0.001$).

Diagnostic performance of single-method assays and dual-/triple-method combinations of Xpert, TB-DNA, and MGIT culture in BALF samples, using the final clinical diagnosis as the reference standard

Using the final clinical diagnosis as the reference standard, we evaluated the diagnostic performance of three single-method assays, namely Xpert, TB-DNA, and MGIT culture, using BALF from patients with smear-negative, newly diagnosed PTB. Among the 142 AFB smear-negative patients, Xpert detected MTB in 86 patients (60.56%), TB-DNA in 104 patients (73.24%), and MGIT culture in 60 patients (42.25%). In the 91 non-PTB patients, Xpert yielded two positive results (2.20%), TB-DNA yielded six (6.59%), and MGIT culture yielded none. Summarized in **Table 4** and **Figure 2D**, TB-DNA showed the highest sensitivity (73.24%; 95% CI: 65.42-79.84), NPV (69.11%; 95% CI: 60.47-76.59), diagnostic accuracy (81.12%; 95% CI: 75.60-85.62), AUC (0.833; 95% CI: 0.780-0.887), and kappa value (0.627) among the three single-method assays. Conversely, MGIT culture recorded the lowest values for these metrics, with sensitivity at 42.25% (95% CI: 34.44-50.48), NPV at 52.60% (95% CI: 45.18-59.90), diagnostic accuracy at 64.81% (95% CI: 58.48-70.65), AUC at 0.711 (95% CI: 0.647-0.776) and kappa value at 0.364; although it achieved 100% specificity and PPV. Compared to Xpert, TB-DNA showed no statistically significant differences in speci-

Molecular assays and MGIT for newly diagnosed PTB

Table 4. Diagnostic performance of single-method assays and dual-/triple-method combinations of Xpert, TB-DNA, and MGIT culture in BALF samples, using the final clinical diagnosis as the reference standard

Diagnostic assay	Clinical diagnosis		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	DA (%)	Kappa value	P value
	SN-PTB (n = 142)	Non-PTB (n = 91)							
Single-method assays									
Xpert								0.527	< 0.001
MTB positive	86	2	60.56 (52.35-68.22)	97.80 (92.34-99.40)	97.73 (92.09-99.37)	61.38 (53.26-68.91)	75.11 (69.18-80.22)		
MTB negative	56	89							
TB-DNA								0.627	< 0.001
MTB positive	104	6	73.24 (65.42-79.84)	93.41 (86.35-96.94)	94.55 (88.61-97.48)	69.11 (60.47-76.59)	81.12 (75.60-85.62)		
MTB negative	38	85							
MGIT								0.364	< 0.001
MTB positive	60	0	42.25 (34.44-50.48)	100 (95.95-100)	100 (93.98-100)	52.60 (45.18-59.90)	64.81 (58.48-70.65)		
MTB negative	82	91							
Various combinations ^a									
Xpert + TB-DNA								0.672	< 0.001
MTB positive	112	8	78.87 (71.44-84.78)	91.21 (83.60-95.48)	93.33 (87.39-96.58)	73.45 (64.64-80.72)	83.69 (78.41-87.88)		
MTB negative	30	83							
Xpert + MGIT								0.609	< 0.001
MTB positive	97	2	68.31 (60.26-75.39)	97.80 (92.34-99.40)	97.98 (92.93-99.44)	66.42 (58.06-73.86)	79.83 (74.21-84.48)		
MTB negative	45	89							
TB-DNA + MGIT								0.674	< 0.001
MTB positive	110	6	77.46 (69.92-83.56)	93.41 (86.35-96.94)	94.83 (89.17-97.61)	72.65 (63.95-79.91)	83.69 (78.41-87.88)		
MTB negative	32	85							
Xpert + TB-DNA + MGIT								0.704	< 0.001
MTB positive	116	8	81.69 (74.52-87.19)	91.21 (83.60-95.48)	93.55 (87.78-96.69)	76.15 (67.34-83.17)	85.41 (80.30-89.37)		
MTB negative	26	83							

Various combinations^a: Both negative results were considered negative, and any positive result was interpreted as positive. PPV, positive predictive value; NPV, negative predictive value; DA, diagnostic accuracy; PTB, pulmonary tuberculosis; SN-PTB, smear-negative PTB; Xpert, Xpert MTB/RIF; MGIT, MGIT culture; BALF, bronchoalveolar lavage fluid; MTB, *Mycobacterium tuberculosis*.

ficity, PPV, NPV, or diagnostic accuracy (all $P > 0.05$). Sensitivity was the only metric that differed significantly between the two assays ($P < 0.05$).

We further evaluated the diagnostic performance of dual-method and triple-method combinations of Xpert, TB-DNA, and MGIT culture in BALF from patients with smear-negative, newly diagnosed PTB. Among the 142 PTB cases, the combinations of Xpert with TB-DNA, Xpert with MGIT culture, TB-DNA with MGIT culture, and the triple-method detected MTB in 112 (78.87%), 97 (68.31%), 110 (77.46%), and 116 (81.69%) cases, respectively. In the 91 non-PTB patients, these four strategies yielded positive results in 8 (8.79%), 2 (2.20%), 6 (6.59%), and 8 (8.79%) cases, respectively. The triple-method achieved the highest sensitivity (81.69%; 95% CI: 74.52-87.19), NPV (76.15%; 95% CI: 67.34-83.17), diagnostic accuracy (85.41%; 95% CI: 80.30-89.37), AUC (0.864; 95% CI: 0.814-0.915), and kappa value (0.704) among the four strategies. Conversely, the combination of Xpert with MGIT culture recorded the lowest values for these metrics, with sensitivity at 68.31% (95% CI: 60.26-75.39), NPV at 66.42% (95% CI: 58.06-73.86), diagnostic accuracy at 79.83% (95% CI: 74.21-84.48), AUC at 0.831 (95% CI: 0.778-0.883), and kappa value at 0.609, although it achieved the highest specificity (97.80%; 95% CI: 92.34-99.40) and PPV (97.98%; 95% CI: 92.93-99.44). Compared with the combination of Xpert with MGIT culture, both the triple-method and the combination of Xpert with TB-DNA showed significantly higher sensitivity (both $P < 0.05$). The combination of TB-DNA with MGIT culture showed no significant difference in sensitivity ($P > 0.05$). Furthermore, no statistically significant differences were observed among the four strategies regarding the specificity, PPV, NPV, or diagnostic accuracy (all $P > 0.05$).

Notably, 2 of 54 cases (3.70%) with very low MTB bacterial loads on Xpert were false positives, alongside six false-positive TB-DNA results. After adjudication by at least two senior tuberculosis specialists and outpatient follow-up exceeding six months, all these discrepant cases showed no evidence of active tuberculosis. Additionally, one MGIT culture-positive case required 106 days for culture-positive, which is considerably longer than the typical

6-week incubation period. In addition, three MGIT culture-positive MTB cases were AFB smear-positive but MPT64-negative; they were initially misidentified as NTM. However, these false-positive NTM results were corrected after the Xpert assay.

Diagnostic performance of single-method assays and dual-method combinations of Xpert and TB-DNA in BALF samples, using MGIT culture as the reference standard

We additionally evaluated the diagnostic performance of Xpert and TB-DNA, both as single-method assays and as the dual-method combination, using MGIT culture as the reference standard. The dual-method combination of Xpert with TB-DNA yielded a sensitivity of 93.33% (95% CI: 84.07-97.38) and an NPV of 96.46% (95% CI: 91.25-98.62), both higher than those of either assay alone. For the single-method assays, Xpert achieved the highest values across several metrics: specificity of 77.46% (95% CI: 70.67-83.05), PPV of 55.68% (95% CI: 45.28-65.61), diagnostic accuracy of 78.54% (95% CI: 72.83-83.33), AUC of 0.796 (95% CI: 0.728-0.863), and kappa value of 0.513 (**Table 5; Figure 2E**). Xpert and TB-DNA showed no statistically significant differences in sensitivity, PPV, NPV, or diagnostic accuracy (all $P > 0.05$). Specificity was the only metric with a statistically significant difference between the two assays ($P < 0.05$).

Discussion

Because some patients cannot produce sputum or provide samples of adequate quality, microbiological testing may yield false-negative results. In such cases, BALF provides an alternative sample that can improve the diagnostic yield for PTB [14]. In this study, the positivity rates of Xpert, TB-DNA, and MGIT culture in AFB smear-positive BALF samples were 100%, 100%, and 92.73%, respectively, substantially higher than the corresponding rates in AFB smear-negative samples (60.56%, 73.24%, and 42.25%, respectively; all $P < 0.001$). This difference likely reflects the lower MTB bacillary burden in smear-negative samples and supports focused evaluation of diagnostic strategies in patients with smear-negative, newly diagnosed PTB.

Molecular assays and MGIT for newly diagnosed PTB

Table 5. Diagnostic performance of single-method assays and dual-method combinations of Xpert and TB-DNA in BALF samples, using MGIT culture as the reference standard

Diagnostic assay	MGIT culture		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	DA (%)	Kappa value	P value
	MTB positive (n = 60)	MTB negative (n = 173)							
Xpert								0.513	< 0.001
MTB positive	49	39	81.67 (70.08-89.44)	77.46 (70.67-83.05)	55.68 (45.28-65.61)	92.41 (86.93-95.71)	78.54 (72.83-83.33)		
MTB negative	11	134							
TB-DNA								0.453	< 0.001
MTB positive	54	56	90.00 (79.85-95.34)	67.63 (60.34-74.15)	49.09 (39.94-58.31)	95.12 (89.77-97.75)	73.39 (67.37-78.65)		
MTB negative	6	117							
Xpert + TB-DNA								0.425	< 0.001
MTB positive	56	64	93.33 (84.07-97.38)	63.01 (55.60-69.84)	46.67 (37.98-55.56)	96.46 (91.25-98.62)	70.82 (64.68-76.28)		
MTB negative	4	109							

PPV, positive predictive value; NPV, negative predictive value; DA, diagnostic accuracy; Xpert, Xpert MTB/RIF; BALF, bronchoalveolar lavage fluid; MTB, *Mycobacterium tuberculosis*.

Rifampicin resistance results from structural alterations in the β -subunit of RNA polymerase encoded by the *rpoB* gene, and approximately 95% of rifampicin-resistance mutations are located within the 81-bp rifampicin resistance-determining region. Xpert uses five fluorescent probes that provide overlapping coverage of the *rpoB* gene, specifically Probe A (codons 507-511), Probe B (511-518), Probe C (518-523), Probe D (523-529), and Probe E (529-533), allowing simultaneous detection of MTB and rifampicin resistance [15, 16]. The TB-DNA assay uses primers and probes targeting the conserved *hsp65* gene of *Mycobacterium* and the MTB-specific *IS6110* gene, thereby enabling differentiation of MTB from NTM. MGIT culture, in turn, shortens the time to culture positivity and improves culture yield compared with Lowenstein-Jensen medium. DNA-based diagnostic methods are valuable for identifying tuberculosis, yet the continued presence of DNA after bacterial death can impede their ability to differentiate viable MTB from nonviable MTB during treatment [17]. Generally, a positive culture suggests viable MTB is present, whereas a negative one means no detectable growth under the tested conditions, though it cannot rule out residual bacterial components or loads below the detection limit [17]. Even if *Mycobacterium* is dormant or nonculturable, it may still have low-abundance but stable mRNA transcripts, indicating that colony formation failure does not necessarily equate to complete transcriptional inactivity [18]. This also indirectly highlights the limitations of MGIT culture as a reference standard. Specifically, TB cases with positive molecular assays but negative MGIT culture may present as false negatives, leading to lower observed specificity for various diagnostic methods.

Using the final clinical diagnosis as the reference standard, we found that the AFB smear positivity rate in BALF samples from patients with newly diagnosed PTB was 27.92% (95% CI: 22.12-34.56), comparable to the 25.2% reported by Bai et al. [19]. In patients with BALF smear-negative, newly diagnosed PTB, Xpert showed a sensitivity of 60.56% (95% CI: 52.35-68.22) and a specificity of 97.80% (95% CI: 92.34-99.40). These values are consistent with the sensitivity of 61.1% and specificity of 96.6% for Xpert in BALF samples reported by Pan et al. [20], and are in alignment with additional published studies [19, 21]. The TB-DNA

assay yielded a sensitivity of 73.24% (95% CI: 65.42-79.84) in BALF smear-negative, newly diagnosed PTB patients, similar to the 75.5% (95% CI: 64.3-86.7) reported by Ruan et al. [22]. Compared with MGIT culture, TB-DNA showed substantial agreement with the final clinical diagnosis, while Xpert exhibited moderate agreement. Both molecular assays achieved significantly higher sensitivity and diagnostic accuracy than MGIT culture (both $P < 0.05$), underscoring the diagnostic superiority of nucleic acid-based methods over conventional culture approaches. Specifically, compared with Xpert, TB-DNA achieved higher values for sensitivity (73.24%; 95% CI: 65.42-79.84), NPV (69.11%; 95% CI: 60.47-76.59), diagnostic accuracy (81.12%; 95% CI: 75.60-85.62), kappa value (0.627), and AUC (0.833; 95% CI: 0.780-0.887), although only the difference in sensitivity reached statistical significance ($P < 0.05$). We observed similar results when using MGIT culture as the reference standard; Xpert and TB-DNA showed no statistically significant differences in sensitivity, PPV, NPV, or diagnostic accuracy (all $P > 0.05$). Specificity was the only metric with a significant inter-assay difference ($P < 0.05$). Collectively, these findings indicate that Xpert and TB-DNA showed broadly similar overall diagnostic performance in BALF from patients with smear-negative, newly diagnosed PTB.

Notably, TB-DNA showed consistently higher sensitivity than Xpert with both reference standards. Previous studies have reported similar findings [22, 23], although contrasting results have also been published [9]. TB-DNA also showed higher sensitivity, NPV, diagnostic accuracy, kappa value, and AUC than the combination of Xpert with MGIT culture. The higher sensitivity of TB-DNA may partly be due to its targeting of *IS6110*, a multicopy element in the MTB genome that facilitates detection of low concentrations of mycobacterial DNA in clinical samples [23]. In addition, the TB-DNA assay incorporates a centrifugation-based concentration step. Its lower cost may also facilitate repeat testing (≥ 2 times) of the same sample. Together, these factors may contribute to the higher sensitivity and related diagnostic metrics observed with TB-DNA.

Among the 143 Xpert-positive patients in this study, 138 (96.50%) were susceptible to rifampicin and 5 (3.50%) were resistant to

rifampicin. Of these 5 resistant patients, 2 were lost to follow-up. The remaining 3 patients received anti-TB treatment for 18 months (2 cases) and 21 months (1 case), respectively, and all were confirmed to be cured at multiple outpatient follow-up visits. Among the 91 non-PTB cases, TB-DNA yielded six false-positive results. This primarily contributed to its lower specificity compared to Xpert. Additional large-scale studies are needed to confirm these observations. Furthermore, our analysis revealed two false positives (3.70%) among 54 cases with very low MTB bacterial loads detected by Xpert. This observation highlights a non-negligible risk of false positivity associated with extremely low MTB burdens. However, patients with negative MGIT culture but positive molecular assay results should not be simply classified as false positives, especially those with high clinical suspicion of tuberculosis. Consequently, clinicians must correlate molecular results with complementary microbiological assays, chest imaging, and the full clinical profile to avoid misdiagnosis. This vulnerability to false positives at low bacterial loads has been corroborated by other studies [24, 25]. Several mechanisms may contribute: (1) Residual DNA from nonviable bacilli may be detected by Xpert [26]; (2) Positive TB-DNA and Xpert results may persist for several years after treatment, even in the absence of culturable mycobacteria [27]; (3) Bronchoscope contamination with MTB may generate false-positive molecular results [28, 29]. Therefore, when Xpert detects a very low MTB burden, the possibility of contamination should be considered, particularly when clinical and imaging findings do not support active tuberculosis [29]. To gain a clearer understanding of the occurrence and clinical relevance of these false-positive results, larger studies are essential.

The MPT64 antigen assay is a rapid immuno-chromatographic method for MTB identification that detects MPT64 antigen in AFB smear-positive cultures and can provide results within approximately 15 min [30]. In our study, the MGIT culture positivity rate among smear-positive PTB patients was 92.73%, which was significantly higher than that among smear-negative PTB patients (42.25%). Like our findings, Qi et al. reported that the average culture positivity time for MGIT culture was 7.45 days in

AFB smear-positive/Xpert-positive samples compared with 14.12 days in smear-negative/Xpert-positive samples ($P < 0.001$), which can be attributed to variations in the MTB bacillary load [31]. Similarly, AFB smear and Xpert positivity, both reflecting higher MTB bacillary burdens, have been associated with shorter times to culture positivity [32]. In our study, MGIT culture showed a sensitivity of 42.25% (95% CI: 34.44-50.48), and specificity of 100% (95% CI: 95.95-100) in BALF samples from patients with smear-negative, newly diagnosed PTB, findings comparable to those reported by Wang et al. [33]. The high specificity observed in our cohort may partly reflect the use of Xpert to further identify AFB smear-positive, MPT64-negative MGIT cultures.

Among the 60 MTB-positive cultures in the smear-negative PTB group, one isolate with a very low MTB bacterial load required 106 days to become culture positivity, a duration that substantially exceeded the conventional 6-week incubation period for MGIT culture. This prolonged time to positivity was consistent with the very low MTB burden detected by Xpert. A multicenter study conducted in Uganda similarly demonstrated a positive association between Xpert cycle threshold (Ct) values and time to culture positivity, indicating that lower bacillary burdens are associated with longer culture detection times [34]. Mahomed et al. also reported that approximately 0.8% of cultures processed using the BACTEC MGIT 960 system became positive after more than 42 days of incubation [35]. In addition, a prospective study in West Africa suggested that prolonged incubation in automated MGIT systems may improve detection in samples with delayed growth [36]. Where laboratory workflow permits, extending the incubation period for samples with low growth indices may therefore help reduce false-negative culture results.

Furthermore, three MGIT culture-positive MTB cases were AFB smear-positive but MPT64-negative. They were initially misidentified as NTM. Subsequent Xpert assay corrected these false NTM results. The 63-bp deletion variant in the MPT64 gene and frameshift mutations in the MPT64 protein following the 3rd, 9th, and 13th amino acids may explain this misidentification [30, 37]. This assay also cannot detect *Mycobacterium bovis* strains with dele-

tions or mutations in the MPT64 gene, which may lead to their misclassification as NTM when using MGIT culture [38]. Therefore, the use of Xpert as a complementary method for identifying MPT64-negative and AFB smear-positive MGIT cultures can substantially reduce, if not prevent, the misinterpretation of MTB-positive cultures as NTM. This finding is further supported by the study of Suresh et al. which demonstrated that Xpert targets the MTB-specific *rhoB* gene [38].

Undoubtedly, the triple-method achieved the highest sensitivity (81.69%; 95% CI: 74.52-87.19), NPV (76.15%; 95% CI: 67.34-83.17), diagnostic accuracy (85.41%; 95% CI: 80.30-89.37), AUC (0.864; 95% CI: 0.814-0.915), and kappa value (0.704) among the four combination strategies evaluated. Sensitivity differed significantly from that of the Xpert with MGIT culture combination ($P < 0.05$), but not from the combinations of Xpert with TB-DNA or TB-DNA with MGIT culture (both $P > 0.05$). Furthermore, no statistically significant differences existed among the four strategies regarding specificity, PPV, NPV, or diagnostic accuracy (all $P > 0.05$). Kappa results demonstrated substantial agreement with the final clinical diagnosis across all combination methods, with all AUC values exceeding 0.83. Although the combinations of Xpert with TB-DNA and TB-DNA with MGIT culture showed comparable diagnostic performance to the triple-method in BALF from patients with smear-negative, newly diagnosed PTB, the triple-method increased sensitivity to 81.69% and diagnostic accuracy to 85.41% while maintaining specificity above 90%. Thus, it remains a feasible strategy to further enhance diagnostic performance.

Wang et al. reported that combining phenotypic methods such as MGIT culture with molecular assays may improve the accuracy of early PTB diagnosis and guide treatment decisions [39]. Nevertheless, Xpert and other molecular assays cannot replace conventional AFB smears, cultures, or phenotypic drug susceptibility tests (DSTs), which remain essential for assessing treatment response and detecting resistance beyond rifampicin [40]. MGIT culture can also increase diagnostic yield when smear or molecular testing is negative and, unlike Xpert, provides evidence of viable myco-

bacteria [35, 41]. Furthermore, isolates obtained from MGIT cultures can be used for phenotypic DST, including assessment of resistance to first- and second-line drugs, as well as additional microbiological characterization [42]. Thus, combining Xpert, TB-DNA, and MGIT culture may provide complementary diagnostic information that supports early diagnosis, treatment selection, and subsequent microbiological evaluation in patients with smear-negative, newly diagnosed PTB.

Our study has several limitations. First, although standardized internal and external quality-control procedures were implemented, residual analytical variability cannot be completely excluded. Second, the single-center retrospective design introduces the possibility of selection bias and limits the generalizability of the findings; larger multicenter studies are therefore needed for validation. Third, classification of false-positive Xpert and TB-DNA results relied primarily on consensus assessment by senior tuberculosis specialists and outpatient follow-up for more than six months, without additional sequencing-based confirmation. Finally, the absence of sputum samples in a subset of patients precluded a comparative analysis of the diagnostic performance of the three methods in BALF versus sputum among newly diagnosed PTB patients.

Conclusion

Xpert showed similar overall diagnostic performance compared to TB-DNA in BALF from patients with smear-negative, newly diagnosed PTB. Notably, the combinations of Xpert with TB-DNA and TB-DNA with MGIT culture demonstrated comparable diagnostic performance to that of the triple-method assay. The triple-method assay integrates two molecular assays with MGIT culture, yielding higher sensitivity and diagnostic accuracy. It thereby supports earlier diagnosis and treatment decision-making and represents a viable strategy for improving diagnostic performance in this patient population.

Acknowledgements

This work was supported by the Zhejiang Provincial Medical and Health Science and Technology Plan (grant No. 2021KY888). We are grateful to our departmental colleagues for

their advice and assistance throughout this study.

Disclosure of conflict of interest

None.

Address correspondence to: Feng Pan, Department of Laboratory Medicine, The Affiliated Hospital of Hangzhou Normal University, No. 126 Wenzhou Road, Hangzhou 310015, Zhejiang, China. E-mail: panfeng1240@126.com

References

- [1] Global tuberculosis report 2025. World Health Organization; 2025.
- [2] Ajantha P, Puri MM, Tayal D and Khalid U. Urinary lipoarabinomannan in individuals with sputum-negative pulmonary tuberculosis. *Indian J Med Res* 2024; 159: 206-212.
- [3] Mnyambwa NP, Ngadaya ES, Kimaro G, Kim DJ, Kazwala R, Petrucka P and Mfinanga SG. Assessment of sputum smear-positive but culture-negative results among newly diagnosed pulmonary tuberculosis patients in Tanzania. *Int J Gen Med* 2017; 10: 199-205.
- [4] Friedrich SO, Rachow A, Saathoff E, Singh K, Mangu CD, Dawson R, Phillips PP, Venter A, Bateson A, Boehme CC, Heinrich N, Hunt RD, Boeree MJ, Zumla A, McHugh TD, Gillespie SH, Diacon AH and Hoelscher M; Pan African Consortium for the Evaluation of Anti-tuberculosis Antibiotics (PanACEA). Assessment of the sensitivity and specificity of Xpert MTB/RIF assay as an early sputum biomarker of response to tuberculosis treatment. *Lancet Respir Med* 2013; 1: 462-470.
- [5] Wang J, Liu LB, Yue YN, Hu YQ, Fan DP, Li H, Zhang Y, Xia Q and Zhu M. Value of RNA isothermal amplification real-time testing and fluorescence quantitative PCR combined with detection of bronchoalveolar lavage fluid in rapid diagnosis of sputum smear-negative pulmonary tuberculosis. *Chin J Nosocomiol* 2017; 27: 300-320.
- [6] Fan L, Li D, Zhang S, Yao L, Hao X, Gu J, Li H, Niu J, Zhang Z and Zhu C. Parallel tests using culture, Xpert MTB/RIF, and SAT-TB in sputum plus bronchial alveolar lavage fluid significantly increase diagnostic performance of smear-negative pulmonary tuberculosis. *Front Microbiol* 2018; 9: 1107.
- [7] Yan HM, Ruan Y, Chen XH, Mao WJ and Chen F. The value of detecting BALF by TB-RNA, TB-DNA and gene chip technology in the early diagnosis of smear-negative pulmonary tuberculosis in children. *J Clin Pulm Med* 2023; 28: 1345-1351.
- [8] Liu Q, Ji Y, Martinez L, Lu W, Shi X, Wang J and Zeng Y. Combined tests with Xpert MTB/RIF assay with bronchoalveolar lavage fluid increasing the diagnostic performance of smear-negative pulmonary tuberculosis in Eastern China. *Epidemiol Infect* 2020; 149: e5.
- [9] Zheng H, Zhong F, Yu G and Shen Y. Comparison of the diagnostic efficacy of the CapitalBio mycobacterium real-time polymerase chain reaction detection test and Xpert MTB/RIF in smear-negative pulmonary tuberculosis. *Eur J Clin Microbiol Infect Dis* 2021; 40: 969-977.
- [10] Liu X, Hou XF, Gao L, Deng GF, Zhang MX, Deng QY, Ye TS, Yang QT, Zhou BP, Wen ZH, Liu HY, Kornfeld H and Chen XC. Indicators for prediction of Mycobacterium tuberculosis positivity detected with bronchoalveolar lavage fluid. *Infect Dis Poverty* 2018; 7: 22.
- [11] Fan L, Guan B, Cheng M, Liu C, Tian Y, Li R and Chen Y. A comprehensive evaluation of a loop-mediated isothermal amplification assay for the diagnosis of pulmonary tuberculosis in children using bronchoalveolar lavage fluid. *Infect Drug Resist* 2022; 15: 975-987.
- [12] Ye N, Ba TE, Yang YL, Yang FM, Wang YJ, Qi M, Ye XL and Luo YQ. Evaluation of assays on bronchoalveolar lavage fluid in early diagnosis of pulmonary tuberculosis. *Int J Clin Exp Med* 2020; 13: 8027-8032.
- [13] National Health and Family Planning Commission of the People's Republic of China. *Diagnosis of Tuberculosis*, WS 288-2017.
- [14] Sabella-Jiménez V, Sabella-Jiménez VL, Restrepo-Espinosa V, Eljadue-Flórez J, Gallardo-Castro CV, Silvera AA, Otero-Herrera C, Arroyo HAM, Acosta-Reyes J and Barrios JLQ. Performance of Xpert MTB/RIF and acid-fast bacilli smear microscopy for the diagnosis of pulmonary tuberculosis using bronchoalveolar lavage samples in negative or sputum-scarce adults in Colombia: a retrospective diagnostic accuracy study. *BMC Infect Dis* 2025; 25: 491.
- [15] Feng G, Jiang H and Chen Y. Efficacy of Xpert MTB/RIF assay in detecting Mycobacterium tuberculosis in samples with different results by smear and culture in a coastal city with high incidence of tuberculosis. *BMC Infect Dis* 2025; 25: 55.
- [16] Nie Q, Sun D, Zhu M, Tu S, Chen N, Chen H, Zhou Y, Yao G, Zhang X, Zhang T, Yang C and Tao L. Phenotypic drug susceptibility characterization and clinical outcomes of tuberculosis strains with A-probe mutation by GeneXpert MTB/RIF. *BMC Infect Dis* 2023; 23: 832.
- [17] Niu J, Zhang P, Wei Z, Zou Y and Wang Z. Beyond culturability: VBNC-like and differentially culturable Mycobacterium tuberculosis challenge growth-based interpretations of bacterial

- viability. *Front Cell Infect Microbiol* 2026; 16: 1896879.
- [18] Ignatov DV, Salina EG, Fursov MV, Skvortsov TA, Azhikina TL and Kaprelyants AS. Dormant non-culturable *Mycobacterium tuberculosis* retains stable low-abundant mRNA. *BMC Genomics* 2015; 16: 954.
- [19] Bai W, Liu L, Wu L, Chen S, Wu S, Wang Z, Xu K, Chi Q, Pan Y and Xu X. Assessing the utility of the Xpert *Mycobacterium tuberculosis*/rifampin assay for analysis of bronchoalveolar lavage fluid in patients with suspected pulmonary tuberculosis. *J Clin Lab Anal* 2022; 36: e24154.
- [20] Pan X, Yang S, Deighton MA, Qu Y, Hong L and Su F. A comprehensive evaluation of Xpert MTB/RIF assay with bronchoalveolar lavage fluid as a single test or combined with conventional assays for diagnosis of pulmonary tuberculosis in China: a two-center prospective study. *Front Microbiol* 2018; 9: 444.
- [21] Le Palud P, Cattoir V, Malbrun B, Magnier R, Campbell K, Oulkhair Y, Zalcman G and Bergot E. Retrospective observational study of diagnostic accuracy of the Xpert® MTB/RIF assay on fiberoptic bronchoscopy sampling for early diagnosis of smear-negative or sputum-scarce patients with suspected tuberculosis. *BMC Pulm Med* 2014; 14: 137.
- [22] Ruan JZ, Wang ZT and Xu SF. Application of GeneXpert MTB/RIF assay in the diagnosis of bacteriologically-negative pulmonary tuberculosis. *Int J Clin Exp Med* 2020; 13: 7766-7772.
- [23] Hemeg HA, Albulushi HO, Ozbak HA, Ali HM, Alahmadi EK, Almutawif YA, Alhuofie ST, Alaeq RA, Alhazmi AA, Najim MA and Hanafy AM. Evaluating the sensitivity of different molecular techniques for detecting *Mycobacterium tuberculosis* complex in patients with pulmonary infection. *Pol J Microbiol* 2023; 72: 421-431.
- [24] Yang CQ, Mei CL, Du RH, Lei M and Qin LX. Analysis of diagnosis and treatment for a pulmonary lymphangitic carcinomatosis misdiagnosed as pulmonary tuberculosis caused by false positive molecular biological test. *Chin J Antituberc* 2022; 44: 289-293.
- [25] Mei CL, Yang CQ, Du RH, Cao TZ, Feng W, Chen SF, Liu XP and Ou JL. Diagnostic accuracy of GeneXpert MTB/RIF in detecting pulmonary tuberculosis with extremely low loads of MTB in bronchoalveolar lavage fluid in general hospitals. *Chin J Antituberc* 2024; 46: 1037-1041.
- [26] Theron G, Venter R, Calligaro G, Smith L, Limberis J, Meldau R, Chanda D, Esmail A, Peter J and Dheda K. Xpert MTB/RIF results in patients with previous tuberculosis: Can we distinguish true from false positive results? *Clin Infect Dis* 2016; 62: 995-1001.
- [27] Theron G, Venter R, Smith L, Esmail A, Randall P, Sood V, Oelfese S, Calligaro G, Warren R and Dheda K. False-positive Xpert MTB/RIF results in retested patients with previous tuberculosis: frequency, profile, and prospective clinical outcomes. *J Clin Microbiol* 2018; 56: e01696-17.
- [28] Ramsey AH, Oemig TV, Davis JP, Massey JP and Török TJ. An outbreak of bronchoscopy-related *Mycobacterium tuberculosis* infections due to lack of bronchoscope leak testing. *Chest* 2002; 121: 976-981.
- [29] Bagrecha M, Ganta SVA and Mirza S. Navigating diagnostic pitfalls: false positivity in GeneXpert *mycobacterium tuberculosis*/rifampicin assay. *Cureus* 2024; 16: e62889.
- [30] Singh K, Kumari R, Tripathi R, Gupta A and Anupurba S. Mutation in MPT64 gene influencing diagnostic accuracy of SD Bioline assay (capilia). *BMC Infect Dis* 2019; 19: 1048.
- [31] Qi JY and Fan W. Study on the value of molecular biology combined with liquid MGIT culture method in clinical examination of *Mycobacterium tuberculosis*. *Am J Transl Res* 2021; 13: 9757-9763.
- [32] Vongthilath-Moeung R, Poncet A, Renzi G, Schrenzel J and Janssens JP. Time to detection of growth for *Mycobacterium tuberculosis* in a low incidence area. *Front Cell Infect Microbiol* 2021; 11: 704169.
- [33] Wang L, Shi JW, Chen XL, Qin ZH, Zheng H and Tang JM. Value of bronchoalveolar lavage fluid effector T-cell assay for the rapid diagnosis of smear-negative pulmonary tuberculosis. *J Clin Pulm Med* 2019; 24: 588-591.
- [34] Najjingo I, Muttamba W, Kirenga BJ, Nalunjogi J, Bakesiima R, Olweny F, Lusiba P, Katamba A, Joloba M and Ssengooba W. Comparison of GeneXpert cycle threshold values with smear microscopy and culture as a measure of mycobacterial burden in five regional referral hospitals of Uganda - a cross-sectional study. *PLoS One* 2019; 14: e0216901.
- [35] Mahomed S, Dlamini-Mvelase NR, Dlamini M and Mlisana K. Failure of BACTEC™ MGIT 960™ to detect *Mycobacterium tuberculosis* complex within a 42-day incubation period. *Afr J Lab Med* 2017; 6: 537.
- [36] Ofori-Anyinam B, Kanuteh F, Agbla SC, Adetifa I, Okoi C, Dolganov G, Schoolnik G, Secka O, Antonio M, de Jong BC and Gehre F. Impact of the *mycobacterium africanum* West Africa 2 Lineage on TB diagnostics in West Africa: Decreased sensitivity of rapid identification tests in the Gambia. *PLoS Negl Trop Dis* 2016; 10: e0004801.
- [37] Song Z, He W, Pei S, Zhao B, Cao X, Wang Y, He P, Liu D, Ma A, Ou X, Xia H, Wang S, Liu C and Zhao Y. Association of lineage 4.2.2 of *Myco-*

- bacterium tuberculosis with the 63-bp deletion variant of the mpt64 gene. *Microbiol Spectr* 2023; 11: e0184223.
- [38] Suresh P, Biswas L, Prasad V, Kumar A, Sivasadas S, Khan S and Biswas R. BCG infection due to MPT64-negative strain: a diagnostic challenge. *Am J Trop Med Hyg* 2020; 103: 1072-1075.
- [39] Wang X, He C, Liu M, Zhang H, Xie J, Zhao X and Meng A. Diagnostic value of phenotypic testing combined with molecular biology testing for tuberculosis. *Sci Rep* 2026; 16: 8692.
- [40] Agrawal M, Bajaj A, Bhatia V and Dutt S. Comparative study of GeneXpert with ZN stain and culture in samples of suspected pulmonary tuberculosis. *J Clin Diagn Res* 2016; 10: DC09-DC12.
- [41] Zhao J, Pu D, Zhang Y, Qu J, Lu B and Cao B. Comparison of performances of GeneXpert MTB/RIF, Bactec MGIT 960, and Bactec Myco/F systems in detecting *Mycobacterium tuberculosis* in biopsy tissues: a retrospective study. *Microbiol Spectr* 2023; 11: e0141422.
- [42] Tyrrell FC, Budnick GE, Elliott T, Gillim-Ross L, Hildred MV, Mahlmeister P, Parrish N, Pentella M, Vanneste J, Wang YF and Starks AM. Probability of negative *Mycobacterium tuberculosis* complex cultures based on time to detection of positive cultures: a multicenter evaluation of commercial-broth-based culture systems. *J Clin Microbiol* 2012; 50: 3275-3282.